REACTIVITY

OF THE

NASAL RESPIRATORY MUCOSA

[A CLINICAL AND EPIDEMIOLOGICAL STUDY]

N. J. GROBLER

REACTIVITY OF THE NASAL RESPIRATORY MUCOSA

"WHAT IS YOUR NOSE FOR?

To breathe with, of course; and to smell with.
But that's far from the whole story"....

(BAUER)

STELLINGEN

I

Veranderingen in het drukverval tussen nasopharynx en buitenlucht gemeten bij gesloten mond tijdens rustademhaling vormen een goede maat voor de reactie van het neusslijmvlies.

II

De patient met spondylitis ankylopoëtica (M. Bechterew) en beperkte intercostale excursie neigt niet tot verhoogde respiratore infecties.

III

Shock, optredend bij patienten met een recent myocard infarct, dient onder meer te worden behandeld met een snel werkend digitalis preparaat; ook indien geen andere tekenen van decompensatio cordis aanwezig zijn.

IV

Provocatie van neusslijmvlies of bronchiaalboom met allergenen, heeft geen zin als de huidreacties t.o.v. deze allergenen negatief zijn.

V

Het is niet zeker, dat bij het „alveolo-capillaire block" syndroom stoornissen in de bloedgassen het gevolg zijn van een verdikking van de alveolo-capillaire membraan.

VI

In het ziekenhuis geboren baby's van moeders die in de zwangerschap rubeola hebben doorgemaakt behoren na de geboorte geïsoleerd te worden verpleegd.

VII

De erfelijke vorm van chronische recidiverende pancreatitis gaat slechts bij een beperkt aantal families gepaard met een verhoogde uitscheiding in de urine van cystine en de di-basische aminozuren en wordt dan waargenomen bij een deel van de patienten en hun gezonde familieleden.

VIII

Alleen wanneer een ventilerende long geen koolzuur uitscheidt, is het zeker dat het pulmonale capillair bed van de betreffende long niet met bloed wordt doorstroomd.

IX

Het is van belang, dat bij kinderchirurgie, diathermie gebruikt wordt voor de bloedstelping.

X

Ofschoon bij de pharmacologische proeven omtrent het ontstaan van focale necrose in het myocard t.g.v. isoprenaline en orciprenaline, hoge doseringen gebruikt werden, zal men klinisch bij het voorschrijven van deze sympathicomimetica toch voorlopig rekening moeten houden met de uitkomsten van deze proeven - zeker indien er kans is op hypoxie van het myocard.

XI

Bij patienten die een tracheotomie hebben ondergaan dient röntgenologisch gecontroleerd te worden of de ingebrachte canule de juiste lengte en kromming heeft.

XII

De resultaten van experimentele atheromatose, bij met cholesterolrijke gevoerde konijnen, mogen niet zondermeer worden betrokken op arteriosclerose bij de mens.

XIII

De gunstige werking van tetracycline bij acne vulgaris berust op een verandering in de samenstelling van het sebum.

XIV

Bepaalde neusklachten berusten ondermeer op een verhoogde reactiviteit van het neusslijmvlies.

XV

Door bepaling van L-ketens is het mogelijk de macroglobulinaemie van Waldenström te onderscheiden van de reactieve vormen (van macroglobulinaemie).

XVI

Het is zeer onwaarschijnlijk, dat de nucleus reticularis thalami de "final common pathway" van de reticulaire formatie van de hersenstam vertegenwoordigt.

XVII

Voor de bezinning inzake de verhouding - geloof en wetenschap, is Psalm 8 van grote betekenis.

N. J. GROBLER
14 september 1966

RIJKSUNIVERSITEIT TE GRONINGEN

REACTIVITY
OF THE
NASAL RESPIRATORY MUCOSA
[A CLINICAL AND EPIDEMIOLOGICAL STUDY]

PROEFSCHRIFT

ter verkrijging van het doctoraat in de geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Mr. E. H. s' Jacob,
hoogleraar in de faculteit der rechtsgeleerdheid,
in het openbaar te verdedigen op woensdag 14 september 1966
des namiddags te 4 uur

door

NICOLAAS JOHANNES GROBLER
geboren te Thabazimbi (Zuid-Afrika)

1966
DRUKKERIJ VAN DENDEREN
GRONINGEN

Promotores:

Prof. Dr. N. G. M. ORIE

Prof. Dr. P. E. HOEKSEMA

This study was supported by grants from the National Research Council TNO, The Hague, The Netherlands; the European Community of Coal and Steel, Luxemburg and Philips-Duphar, Weesp, The Netherlands.

Publication of this thesis was made possible by grants from The Netherlands Asthma Foundation* Utrecht; the Groningen University Fund** and the Royal Netherlands Tuberculosis Association, The Hague.

* Stichting het Nederlands Astma Fonds.
** Stichting Groninger Universiteitsfonds.

To Olga

This thesis was prepared in the Allergy Laboratory (Head: K. de Vries, M.D.) of the Pulmonary Division (Head: Professor N. G. M. Orie, M.D.), Department of Medicine (Head: Professor E. Mandema, M.D.) and partly in the Department of Oto-Rhino-Laryngology (Head: Professor P. E. Hoeksema, M.D.), of the State University Hospital, Groningen (The Netherlands), for the Degree of Doctor of Medicine.

INDEX

INTRODUCTION

The experience acquired in studying the pathophysiological mechanisms which result in the manifestation of bronchial obstruction, formed the occasion to perform similar investigations on the nose.

Bronchial narrowing is attributable to at least two different mechanisms: *allergy* - based on an antigen-antibody reaction, and *hyperreactivity* - which can be described as an increased susceptibility to different non-antigenic stimuli, e.g. chemical and physical agents.

Another, sometimes important factor, is the mechanical factor: a complex - consisting of the elasticity of the lung tissue, rigidity and collapsibility of the bronchial wall respectively.

In the evaluation of our results of these clinical experiments with allergens, chemical and physical agents, the localization of the site of action of these stimuli in the bronchial tree, namely, bronchial muscle or bronchial mucosa, formed a challenge.

The differentiation, however, of these possible components by means of a simple clinical experiment is rather difficult.

We considered that the reaction pattern of the nasal respiratory mucosa could possibly be a "model" for the reaction pattern of the respiratory mucosa of the lower respiratory tract. The rationale of this suggestion is supported by:

— a certain resemblance of reaction of the nose and lower respiratory tract, and
— the co-existence of nasal affections ("vasomotor rhinitis" and pollinosis) and Chronic Non-Specific Lung Disease (CNSLD) as described in the literature.

In this investigation, special attention has been paid to the aspecific excitability of the nasal respiratory mucosa - on the model of the experiments done concerning the aspecific excitability of the bronchial tree in patients with CNSLD. The experience gained by these experiments, can be outlined as follows.

1. In patients with CNSLD an increased susceptibility of the bronchial tree to histamine and acetylcholine exists, in comparison with normals (CURRY, TIFFENEAU, DE VRIES).
2. An association exists between the effect of non-allergic stimuli (e.g. SO_2, cold air and fog) and that of histamine and acetylcholine on the bronchial tree. Therefore, the reaction of the bronchial tree to histamine or acetylcholine, has been accepted as an index value of the bronchial aspecific excitability.
3. Whether a definite pathophysiological mechanism can be held responsible for this aspecific bronchial excitability, is unknown.

The object of this investigation was to investigate whether the nasal respiratory mucosa reacts in a similar manner as the bronchial tree does to exogenous stimuli, viz. specific (allergens) and non-specific agents; possibly the results could be of importance for a better conception of the reactivity.

In the first chapter, a brief review of the normal anatomy and physiology of the nose will be given, while in chapter II the clinical picture of pollinosis and "vasomotor rhinitis" will be considered.

In chapter III, the method devised for the assessment of the reaction of the nasal respiratory mucosa to stimuli will be discussed as well as the criteria necessary for their assessment.

The clinical significance of the histamine reactivity of the nasal respiratory mucosa will be considered in chapter IV with reference to an investigation done in a group of patients and in two random samples of "normal" populations.

In chapter V, the results will be given concerning the effect of certain pharmacological agents on the nasal respiratory mucosa.

In chapter VI, a comparative investigation concerning the effect of histamine and allergens on the nasal respiratory mucosa and the bronchial tree will be represented while in chapter VII the results of an investigation regarding the effect of non-specific (non-allergic) stimuli on the nasal respiratory mucosa will be given.

In the last chapter (chapter VIII) a general discussion of the results and the final conclusions will be given.

Chronic non-specific lung disease (CNSLD) will repeatedly be mentioned in this thesis - therefore, it seems appropriate here to give some remarks concerning the terminology.

Current definition of CNSLD

A patient is considered to be suffering from CNSLD, if he or she shows one or more of the following symptoms:

a. Attacks of dyspnoea or continuous shortness of breath of varying intensity.
b. Coughing and/or expectoration on most days of at least 3 months of the year, for at least 2 consecutive years.

In this investigation a patient is not considered to be suffering from CNSLD when these symptoms co-exist with more explicitly described diseases which either directly or indirectly affect the respiratory tract and preceed the development of symptoms. In this connection, the following affections can be mentioned:

1. Localized lung disease as lung tuberculosis, sarcoidosis (M. BESNIER-BOECK), pneumoconioses, bronchiectasis as the result of corpus alienum, cystic disease, neoplasms, collagen disease, generalized pulmonary fibroses and granulomatosis.
2. Primary cardiovascular-renal diseases.
3. Diseases of the chest wall.
4. Diseases of the upper respiratory tract (e.g. laryngitis, sinusitis).
5. Neuroses, e.g. hyperventilation syndrome.

The purpose of the current definition is to create the possibility of a uniform classification of individuals with similar manifestations, so that comparable categories can be formed (Committee of the Health Council of The Netherlands). It is particularly important for epidemiological investigations and it only serves a useful purpose if, for any individual or a group of patients, as many objective data are added as possible.

Chapter I

ANATOMY AND PHYSIOLOGY OF THE NOSE

§ 1. **Applied anatomy**

For purposes of description the nose can be divided into the
I. External nose
II. Internal nose

I. *External nose**

The external nose can vary considerably in size and shape. It is pyramidal in form and covered by skin, muscles and subcutaneous tissue. It can be divided into:

a. bony pyramid
b. cartilaginous vault
c. lobule

a. *The bony pyramid* is formed by the
1. nasal processes of the maxilla
2. nasal bones

The latter articulate with the nasal process of the maxilla, the nasal process and the nasal spine of the frontal, the perpendicular plate of the ethmoid, and with one another. The medial surfaces of the nasal bones project into the nose to form crests which are part of the nasal septum. The nasal bones may differ in size and even in number or be completely absent congenitally.

b. *The cartilaginous vault* is formed by the
1. upper lateral cartilages
2. part of the septal cartilage

* Some data obtained from Cottle.

Proximally, the two lateral cartilages are continuous with each other as well as with the septum.

The proximal border of the cartilage vault lies under the distal border of the nasal bones with which there is firm apposition. The caudal rim of the lateral cartilage is extremely thin and the terminal portions of these cartilages lie under the upper edges of the lobular cartilages.

c. *The lobule* consists of the

1. lobular cartilages
2. tip
3. alae
4. columella
5. membranous septum

The lobular cartilages with their lateral and medial crura are normally concave viewed from within.

Each half of the lobule encircles the beginning of its corresponding nasal chamber, the vestibule.

II. *Internal nose*

The internal nose is usually described as that part which contains the conchae and the septum. The septum which is osseous dorsally cartilaginous ventrally and cutaneous at the apex of the nose, divides the nasal cavity into two chambers which are alike in reverse fashion. The nasal chambers extend from the nares (nostrils) anteriorly to the nasal apertures or choanae posteriorly, where they communicate with the nasopharynx.

Each nasal chamber can be divided into the vestibule, the atrium, the olfactory and the respiratory regions.

The vestibule is the expanded portion within the anterior aperture of the nose. It is bounded laterally by the ala, medially by the mobile septum and columella, proximally by the limen and ostium internum, distally by the nostril.

The so-called nasal valve is formed at the "junction" of the upper lateral cartilage with the septum. The thin and delicate terminal portion of the upper lateral cartilage lies under the upper edge of the lobular cartilage. It is not attached to the septum, so it can move to and from the septum during respiration.

The upper region of the vestibule extends into the atrium, which is marked by a depression on the lateral wall anterior to the upper end of the middle concha. Above, the atrium is defined by a ridge of mucous membrane, the agger nasi.

The olfactory region consists of the upper part of the nasal chamber and is related to the superior concha and the upper part of the septum. The cribriform plate of the ethmoid bone forms its roof. The remainder of the nasal chamber is the respiratory portion.

The lateral wall of the nasal chamber is composed of a number of bones and presents a complicated form owing to the presence of three scroll-like projections, the superior, middle and inferior conchae (turbinate bones). The spaces overhung by the conchae are the meatuses. The small superior meatus shows an opening which leads into the posterior ethmoidal sinus, the middle meatus contains the ostia to the frontal sinus, the anterior ethmoidal sinus and the maxillary sinus (antrum of Highmore). Near the anterior end of the inferior meatus is the opening of the nasolacrimal duct.

§ 2. Histology of the nasal mucous membrane

Histologically, the nasal mucous membrane can be divided into the:

a. vestibular region
b. olfactoray region
c. respiratory region

a. The *vestibular region* is lined by stratified squamous epithelium resting upon a connective tissue corium. In the anterior part the epithelium has a superficial horny layer with stiff hairs (vibrissae) and many sebaceous glands. In the posterior part the superficial keratinized layer, glands and hairs are absent.

b. The *olfactory region* is confined to the superior concha and the upper one third of the septum. The mucous membrane in this region is yellowish in colour. It is lined by a thick pseudostratified non-ciliated columnar epithelium, composed of olfactory receptor cells, supporting cells and basal cells. In the olfactory mucosa numerous tubulo-alveolar glands are found, the secretion of which bathes the surface of the mucosa.

c. *The respiratory region*

A highly specialized tissue forms a continuous lining for the entire respiratory tract. This extends from the remaining part of the nasal cavity and the accessory sinuses to the bronchioles. It is formed by a pseudostratified columnar ciliated epithelium. Among the ciliated cells, many mucus-containing goblet cells are found. The intervening spaces are filled by irregular supporting cells. A fibro-elastic tunica propria underlies this epithelium.

The highly vascular sub-epithelial layer consists of loose cellular connective tissue in which numerous glands and small aggregations of lymphoid tissue are found. In the upper respiratory tract the deeper, more fibrous layer of the mucosa is firmly attached to the underlying perichondrium or periosteum. Therefore this layer is called mucoperichondrium or mucoperiosteum.

§ 3. Blood and nerve supply of the nasal mucosa

Blood supply

The nasal mucosa has an abundant blood supply mainly from the sphenopalatine branches of the internal maxillary artery and the anterior and posterior ethmoidal branches of the ophthalmic artery. The sphenopalatine branches mainly supply the conchae, septum and meatuses. The superior portion of the nose is supplied by the anterior and posterior ethmoidal branches. The superior labial artery, a branch of the external maxillary, supplies the lower and anterior portion.

Venous drainage is principally through the sphenopalatine, opthalmic and anterior facial veins.

The architectural arrangement of the nasal blood vessels have been studied by different investigators e.g. LUCAS (1935), SWINDLE (1937), HARPER (1947), DAWES and PRICHARD (1953), BATSON (1954). In man the venous system lies superficial to the arterial system. The veins form a close cavernous plexus under the nasal respiratory mucosa, especially marked over the inferior conchae, the inferior margin and posterior border of the middle conchae and to some extent on the related parts of the septum. On these places, the cavernous plexuses form the so-called "swelling bodies". The erectile tissue in the mucosa is well supplied with elastic tissue and

muscle fibres. LUCAS demonstrated sphincter muscles at the end of the venous sinusoids.

The arterioles, veins and capillaries form complex anastomoses.

The vasomotor nerve supply is derived from the autonomic nervous system.

Nerve supply

The nerve supply of the nasal cavity can be divided into a:

1. sensory nerve supply (afferent)
2. motor nerve supply (efferent)

1. The sensory nerve supply of the nasal mucosa is provided by branches of the first and second divisions of the trigeminal nerve. These are the:

a. *Anterior ethmoidal nerve*

This nerve divides into an internal median and lateral branch on entering the nasal cavity. The former or septal branch supplies the anterior portion of the septum while the latter, after supplying the anterior portion of the lateral wall of the nasal cavity (including the middle and inferior conchae), emerges as the external nasal branch and supplies branches to the skin and fascia of the lower part of the dorsum and the tip of the nose.

b. *Palatine nerves*

These nerves, three in number, pass the palate through the palatine canals. The *greater* palatine nerve separates into many branches for the supply of the soft palate and the mucoperiosteum of the hard palate. In the palatine canal, this nerve gives off nasal branches to the inferior concha. The *lesser* palatine nerves, supply the soft palate and adjacent parts of the tonsil.

c. *Sphenopalatine nerves*

In its course through the nasal cavity, each *long* sphenopalatine nerve furnishes collateral branches to the mucous membrane of the roof and septum while the *short* sphenopalatine nerves are distributed

as small branches to the superior and middle conchae and the postero-superior part of the septum.

d. *Anterior superior dental nerve*

This nerve passes through a sinuous canal to the root of the anterior nasal spine and reaches the wall of the nose at the level of the anterior end of the inferior concha. Passing from here downwards, this nerve near its termination, distributes branches to the lateral wall and floor of the nasal cavity and to the septum.

2. The nasal motor (autonomic) nerve supply can be divided into a *cranial parasympathetic* and a *cervical sympathetic* system. The former is derived from the parasympathetic fibres situated in the pars intermedia of the facial nerve, while the latter comes from sympathetic fibres of the upper thoracic segments of the spinal cord and the superior cervical sympathetic ganglion. These two systems reach the nasal mucous membrane via the sphenopalatine ganglion. (A parasympathetic and sympathetic supply via the anterior ethmoidal nerve, is also suggested by MALCOMSON, 1959).

The *sphenopalatine ganglion* is of considerable clinical importance. Various groups of nerve fibres have an association with this ganglion:

a. sympathetic post-ganglionic fibres from the deep petrosal nerve coming from the superior cervical ganglion via the carotid plexus; these fibres have no synaptic relation with the ganglion;
b. pre-ganglionic parasympathetic fibres emerging in the pars intermedia of the facial nerve via the geniculate ganglion, reach it by way of the greater superficial petrosal nerve, which forms synapses with post-ganglionic neurons; the petrosal nerves join to form the nerve of the pterygoid canal (Vidian nerve).
c. fibres derived from the maxillary nerve which have no relation with the ganglion other than one of contiguity.

§ 4. Nasal reflex pathways

These have a similar pattern to other reflex pathways in the body and can be divided into the nasal afferent and efferent pathways:

a. *nasal afferent* pathways are mainly trigeminal. Autonomic afferent pathways have also been reported by various investigators, e.g. CHRISTENSEN (1934), KUNTZ (1934), ZIEGELMAN (1934), GOLDING-WOOD (1961);
b. *nasal efferent* pathways travelling to the nasal mucosa are derived from the cranial parasympathetic and the cervical sympathetic systems.

a. The trigeminal nerve fibres arising between the epithelial cells of the nasal mucosa have their cells in the semilunar ganglion. The central connection of these fibres is located in the pons, viz. the sensory terminal nucleus of the trigeminal, which consists of an enlarged upper end (main sensory nucleus) and an elongated lower portion (nucleus of the spinal tract V). The nucleus of the spinal tract V, passes through the pons and medulla and becomes continuous with the dorsal part of the posterior column of gray matter of the spinal cord. The cells of the sensory nucleus of the fifth nerve with their processes, form the neurons of the second order in the afferent reflex pathway from the nasal mucosa. The centrally directed processes are spoken of as the trigemino-thalamic tract, which passes to the cerebral cortex. The fibres of the trigemino-thalamic tract decussate to form an opposite ascending tract to the thalamus. A few fibres of the trigeminal tract ascend uncrossed to the thalamus. Before entering the thalamus, fibres are given off to the reticular formation of the brain stem, which plays an important rôle in the vegetative regulation. In the thalamus, synapses are made with the cortical neurons.

b. The efferent cerebrospinal axis is mainly connected with the nasal mucosa by means of two orders of neurons: the first consists of the pre-ganglionic neurons which have their cell bodies within the brain (or spinal cord). Their peripheral axons, which end by synapsing within the autonomic ganglia, are connected with cell bodies of the neurons of the second order — the post-ganglionic fibres. The peripheral axons of these post-ganglionic fibres pass to the nasal mucosa as parasympathetic (action: vasodilator) and sympathetic (action: vasoconstrictor) fibres via the sphenopalatine ganglion.

§ 5. Respiratory nasal function

In connection with the investigations described in the following chapters, a short review of the respiratory functions of the nose will be given.

Air conditioning

The nose serves as a natural respiratory pathway. It provides protection for the tracheobronchial tree and the delicate pulmonary alveolar epithelium from extreme variations of temperature and humidity. Special heating and moistening of the inspired air are significant functions of the nasal mucosa. No matter what the temperature of the air may be before it is inhaled, it is raised or lowered, as the case may be, to near the body temperature. Intimately associated with the process of heating is that of moistening, since both depend upon the blood supply.

Structurally, the nasal chambers with a relatively large area of respiratory mucous membrane, cavernous vascular bed and abundant glandular secretion, are well adapted to fulfil this vital function. The mucous membrane of the lower respiratory tract and the epithelial walls of the alveoli are thus protected against variations in temperature and humidity. Usually, when the air is raised in temperature, the capacity to absorb moisture is increased. With the engorgement of the erectile tissue, the nose gives off moisture which is taken up by the expanded air and transferred to the lower respiratory tract. This is of considerable importance since, to function normally the lower respiratory tract must be kept moist.

Failure of the humidifying function is associated with drying of the mucosa of the pharynx, larynx, trachea and bronchi. SLOME pointed out that drying of the larynx and the tracheobronchial tree results in accumulation of strings of ropy mucus and inspissated secretions. This is followed by hyperplasia and reduced resistance to infection. The nasal chambers ensure nearly full saturation of the inspired air. The inspired air becomes 75-95 % saturated in the nasal cavity. The volume necessary to saturate the total daily pulmonary ventilation depends on the temperature and relative humidity of the atmosphere. The daily nasal volume of secretions and transudate is about 1 litre, from which about 700 ml is used for saturating the inspired air (SLOME).

Humidification of inspired air in the oral cavity is effective only over short periods of time; prolonged mouth breathing results in a marked drying of the mouth and pharynx.

Protective filtration and ciliary mechanism

The protection of the pulmonary alveolar epithelium from foreign particles in the inspired air, is carried out in the nose by hairs and the mucus film.

Coarse bodies, e.g. insects, are filtered out by the vibrissae in the vestibule. The inspired air, passing the nasal chambers in curved pathways, comes in an intimate contact with the moist mucous membrane. In this way bacteria, dust and other small particles are deposited. These foreign substances are retained by the mucus blanket and conveyed to the pharynx by the continuous action of the cilia.

Regulation and resistance

The regulation and the creation of respiratory resistance is accomplished chiefly by the narrow nasal valve area and by the cavernous tissue of the turbinates. The reflex regulation of the nasal resistance effectively regulates the flow of air to the alveoli. Pressure differences are thus created between the lungs and the nostrils, and in this way contribute to the flow of air.

According to COTTLE, the nose provides almost half of the necessary resistance to air currents. SLOME, however, reported that the nasal chambers contribute probably only 30 per cent of the total resistance.

Among others, VAN DISHOECK, DE WIT and PROETZ contributed to a better understanding of the mechanism by which the nose alters resistance to air flow.

§ 6. Pathophysiology of nasal obstruction, nasal hypersecretion and sneezing

As these symptoms will be encountered in the following chapters some remarks will be given here regarding their pathophysiological background.

Nasal obstruction

EGGSTON and WOLFF explained the congestion of the nasal mucosa as the result of a simultaneous dilatation of the arterioles and constriction of the venous channels. In this way the venous spaces may be readily filled, resulting in an erectile condition of the nasal respiratory mucosa. These investigators pointed out that in different decades of life there exist variations in the vascular supply of the nasal mucosa: in old people some atrophy of the structures occurs with reduced vascular supply in the cavernous spaces of the conchae and septum.

Different clinical studies confirmed that section of the cervical sympathetics (Horner's syndrome) results in swelling of the nasal mucosa, hypersecretion and sneezing attacks [STEINMANN (1948), BEICKERT (1950), STOKSTED and THOMSEN (1953)]. On the other hand, resection of the greater superficial petrosal nerve results in a shrunken nasal mucosa, with a decreased secretion on the homolateral side [GARDNER et al. (1947), MALCOMSON (1959), GOLDING-WOOD (1963)].

The system for engorgement and depletion is under reflex nervous control (PROETZ, 1943).

Nasal secretion

To maintain a constant moist surface the nasal respiratory mucosa is richly supplied with goblet cells and numerous sero-mucous glands. Increase of goblet cells occurs, according to PROETZ, under adverse circumstances. NEGUS found an abnormal increase of goblet cells in the nasal mucosa of asthmatic patients. Mucus from the goblet cells and sero-mucous glands forms a lubricating and protecting blanket for the mucosa of the nose in the same way as it does in the whole respiratory tract.

The nasal mucus is composed of mucin (about 3 %), water (95-97 %) and salts (1-2 %). Mucin, a complex polysaccharid combined with a protein, gives the mucus viscosity and stickiness, so that the secretions are highly adherent to the cilia.

The average pH of nasal secretions ranges from 7.6-8.4. The nasal mucous glands are controlled by the autonomic nervous system. An increased secretion by parasympathetic stimulation was demonstrated among others by MALCOMSON and GOLDING-WOOD.

In addition, vascular transudation for the supply of fluid has also been reported [FLOREY et al. (1932), NEGUS (1957)].

Sneezing

Sneezing can be described as a sudden expiration with a wide open glottis, preceded by one or more inspiratory efforts. With the onset of the inspiration, a rapid rise of intrapulmonary pressure occurs which at a certain value, suddenly forces air into and through the nasal cavity.

It is impossible to sneeze voluntarily - so sneezing is a reflex being controlled by the autonomic (parasympathetic) nervous system.

A stimulus (e.g. chemical, physical or mechanical), adequate to excite the peripheral nerve endings in the nasal respiratory mucosa, results in sneezing accompanied by vasodilatation and increased secretion.

Sneezing can also be triggered by afferent impulses from the eyes, e.g. a sudden look into bright light (BRAUS, VON CURT ELZE, 1960).

§ 7. Reflexes from the upper respiratory tract

In this thesis, the relation between nasal complaints and affections of the lower respiratory tract will be repeatedly discussed. Therefore, a brief review will be given regarding changes in the bronchial tree following upper respiratory tract irritation.

Already in 1844, HERCK emphasized a "nasal factor" in asthma. KRATSCHMER (1870) reported airway changes in the lower respiratory tract as a result of irritation of the nasal mucosa, while VOLTOLINI (1880) reported a cure of asthma following the removal of a nasal polyp. In 1900, ADAM also suggested nasal irritation as a cause of asthma. DIXON and BRODIE (1903) and DIXON and RANSON (1912) experimentally produced bronchoconstriction by stimulation of the nasal respiratory mucosa. Most sensitive is the upper posterior part of the septum — and this, has been called the "asthmagenic area". It has been suggested that deflected septa, spurs or polyps, by pressing upon this area, may cause a reflex effect which results in bronchoconstriction (BRUBAKER, SLUDER, 1919).

SLUDER (1919) reported that application of horse serum in the

region of the sphenopalatine ganglion provoked an asthmatic attack. When the horse serum was applied on any other part of the nasal mucosa only sneezing, swelling of the mucosa, and increased secretion resulted.

Recently, NADEL and WIDDICOMBE (1962), SLOME (1965) and WIDDICOMBE (1966), reported that stimulation of the nasal mucosa by different stimuli, e.g. physical, chemical or mechanical, results in a constriction of the lower respiratory tract. "A reflex initiated in nasal receptors by irritants can cause apnea...." (COMROE, 1965).

Chapter II

POLLINOSIS ("HAY FEVER") AND "VASOMOTOR RHINITIS"

§ 1. Pollinosis

1. *Historical Note*

GALENUS A. D. 200, BOTALLUS (1565), BINNINGERUS (1673), LEDELIUS (1683), and other physicians of Antiquity and of the Middle Ages were well aware of the fact, that certain persons are seized by sneezing attacks and itching in the presence of certain plants (e.g. roses, lilies, flowers of grasses).

Later, early in the last century (1819) JOHN BOSTOCK (homeopath) reported his own symptoms in a paper entitled: "case of a periodical affection of the eyes and chest". In 1828, BOSTOCK published another communication: "catarrhus aestivus" ("summer catarrh") in which he first employed the term "hay fever". BOSTOCK described this "summer catarrh" as a disease with a definite symptomatology with a seasonal occurrence. In the same year MCCULLOCH (1828) wrote that the disease was caused by "hay fields". GORDON (1829), first mentioned "hay asthma" and ascribed the difficulty in breathing to the aroma of vernal grass.

In 1831, ELLIOTSON reported that the clinical symptoms were attributable to the pollens of grasses and not to hay. SALTER (1864), stressed the association of the symptoms with the hay season and in 1862, PHOEBUS approved the "summer catarrh" of BOSTOCK.

BLACKLEY in 1873, proved with experiments on himself that "hay fever" is due to pollen. He evoked "hay fever" manifestations in predisposed individuals by sniffing the "dust" of grass blossoms and succeeded in showing that application of pollen to a scarified skin resulted in oedematous swelling and itching. BLACKLEY thus became the first to perform skin tests.

Later, DUNBAR (1903), confirmed the observations reported by

ELLIOTSON and BLACKLEY. He suggested that the pollen protein possessed a toxic property and injected animals with the so-called "toxalbumin" to obtain an antigenic serum "pollantin" to immunize patients passively. WOLFF-EISNER in 1906 and WEICHARDT in 1907 regarded "hay fever" as a special instance of human hypersensitiveness to pollen protein. Furthermore, NOON (1911), PRAUSNITZ (1930), BENJAMINS (1934), FEINBERG and STEINBERG (1933) repeatedly demonstrated that some pollen grains are sufficient to cause an attack of "hay fever".

BENJAMINS (1931) devised a special method to divide the different components of pollen and concluded that different substances are present in the pollen — large molecular (protein) and smaller molecular (unknown) components responsible for the annual recurrence of the symptoms in the pollinosis patient.

2. *Aetiology*

Three factors are essential for the development and manifestations of pollinosis, viz. the inheritance of a capacity to become sensitized, the sensitization itself and adequate exposure to the (grass) pollen to which the subject has become sensitive.

3. *Symptomatology of pollinosis*

A characteristic feature of pollinosis is the seasonal occurrence. The nasal and conjunctival symptoms usually start within a few days after the beginning of the blooming season of the plant involved. The onset may be gradual or sudden. Usually, however, attacks of pollinosis are of sudden onset. When the onset is gradual, the attack is usually preceded by a mild sensation of itching in the eyes and palate.

In most cases the clinical picture is dominated at first by nasal manifestations. Along with tickling or itching sensations in the soft palate and nose, the patient experiences a distressing urge to sneeze which is usually followed by paroxysms of sneezing accompanied by increased clear watery secretion of the eyes and nose, and nasal obstruction. These manifestations are frequently accompanied by headache, a feeling of heaviness in the head, sometimes photophobia and mental depression.

4. a. *Influence of age*

SCHEPPEGRELL (1922) reported his results regarding the influence of age and sex in 1000 "hay fever" sufferers and concluded that the most common period of the development of "hay fever" is between the ages of 20 and 40 years (64 %). He further stated that the duration of "hay fever" in these cases varied from one month to 46 years; 50 % of the cases had suffered for over 10 years, 12 % over 20 years, 4 % over 30 years.

HUBER (1931) submitted data of 300 "hay fever" sufferers taken at random and gave the following percentages regarding the onset of "hay fever" symptoms:

23 % in the first decade
35 % in the second decade
26 % in the third decade
12 % in the fourth decade
4 % above the fourth decade.

In 1931, COCA, WALZER and THOMMEN reported that pollinosis has its onset mostly between the ages of 20 and 40 years. HANSEL (1936) stated: "it is generally known that hay fever is much more common among younger individuals". He studied 60 "hay fever" patients and found 35 between the ages of 15 and 30 and 51 between the ages of 15 and 40 years. In 50 of the 60 patients, the onset of "hay fever" occurred before the age of 30 years. HANSEL (1936) further admitted that among 60 adult patients with "hay fever", the duration varied from one year or less to 24 years.

TUFT (1937) stated that - although pollinosis may occur at any age, it is probably most frequent between the ages of 15 and 40 years. FEINBERG (1946) pointed out that most pollinosis sufferers begin to have symptoms in childhood or in early adult life - as a matter of fact he said, "hay fever" seldom begins after 40 years of age. URBACH and GOTTLIEB (1946) took care to point out that pollinosis most commonly has its onset between the ages of 16 and 35 years. Fig. 1 shows the age incidence of "hay fever" in clinical patients according to BRAY (1937), SERAFINI (1950), HANSEL (1953) and VOORHORST (1962).

From these clinical studies, it can be seen that in the majority of instances, pollinosis has its onset between the ages of 15 and 40

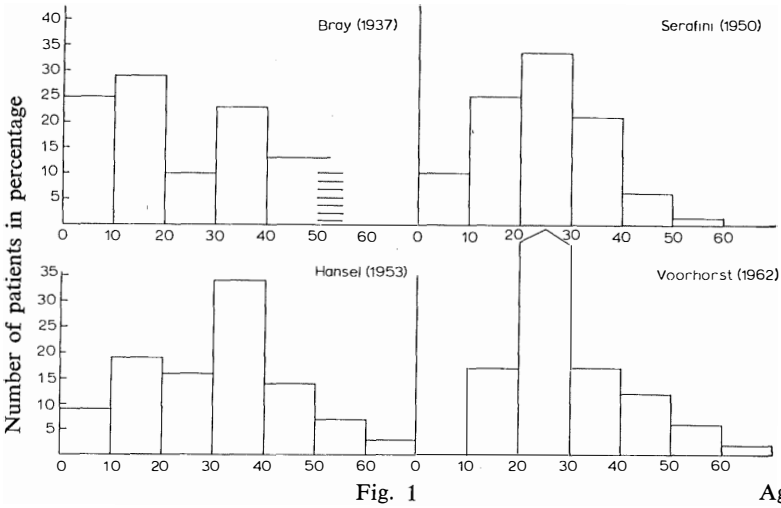


Fig. 1

The age incidence of pollinosis in (clinical) patients according to Bray (1937), Serafini (1950), Hansel (1953) and Voorhorst (1962).
(From Weller, 1965).

years. LOGAN and CUSHION (1958) reported results regarding the prevalence of "hay fever" in a "normal" population and gave the following rate of incidence per 1000 individuals (see fig. 2).

From fig. 2 it can be seen that the greatest frequency of occur-

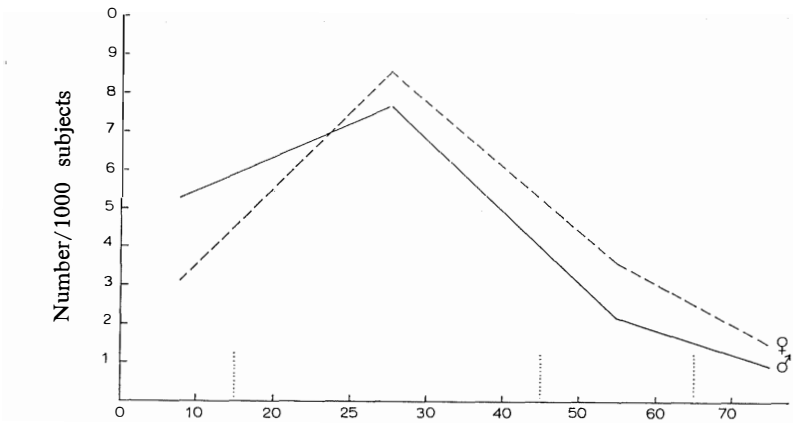


Fig. 2

The prevalence of pollinosis in a "normal" population, according to Logan and Cushion (1958).
(From Weller, 1965).

rence lies between the ages of 15 and 45 years which is in good agreement with the results of the abovementioned clinical studies.

"Hay fever" becomes increasingly rare with increase of age, and according to various investigators there exists a general tendency to decrease after the age of forty [e.g. BRAY (1931), FEINBERG (1946), SERAFINI (1950), HANSEL (1953), QUARLES VAN UFFORD (1954), ORIE et al. (1961), WELLER (1965)].

b. *Influence of sex*

From observations made in 1000 "hay fever" sufferers, SCHEPPEGRELL (1922) encountered 44 % males and 56 % females. However, he admitted that by means of a questionnaire of the United States Public Health Service, it was shown that 63 % of "hay fever" sufferers were males and 37 % females - a difference which he considered as being due to a greater degree of exposure rather than to any difference in susceptibility between the two sexes. COCA, WALZER and THOMMEN (1931) stated that more males are afflicted with this malady than females and quoted figures of many investigators, e.g. PHOEBUS (1862) noted 104 males and 50 females, BEARD (1876) noted 133 males and 67 females while WYMAN (1872) noted 54 males and 25 females.

WOLFF-EISNER (1906) found that the ratio was 3 males to 1 female, while GAREL (1906) found the ratio 3 males to 2 females. COKE (1933) made observations in 400 "hay fever" patients and reported that 211 (53 %) were males and 189 (47 %) were females. He concluded that the sexes are very similarly affected. TUFT (1937) said: "sex appears to have no influence, male and female being affected in the same ratio". FEINBERG (1946), HANSEL (1953), ALBERT (1960) and JAMES (1965) are of the same opinion that the male and female groups are comparatively equally affected.

5. *Clinical course*

The clinical course of pollinosis is variable. The character, duration and intensity of the manifestations are greatly dependent upon the concentration of pollen in the atmosphere and the degree of sensitivity. Symptoms in most pollinosis sufferers are most marked

in the morning (greatest pollen concentration in the morning) [DURHAM (1928), TUFT (1937) and ALBERT (1960)]. Rain fall or humidity tends to decrease pollination while sunshine (stimulates the anthers to open) and high winds favour pollination with consequently manifestations of pollinosis.

Pollinosis symptoms persist for the length of the pollination period of the plant involved and usually recur every year. The seasonal date of onset is fairly constant for most pollinosis sufferers, however, factors influencing pollination will likewise affect the manifestations of pollinosis. In some patients the onset may be early in the season and in other much later. In some patients the onset and termination of symptoms may be sudden and abrupt respectively, while in other individuals a more gradual onset and termination may be the case.

6. *Associated manifestations*

a. Asthma
b. Urticaria
c. Systemic

ad a. *Asthma*

GORDON in 1829, ascribed the seasonal difficulty in breathing to the blooming vernal grasses and called this seasonal difficulty to breathe - "hay asthma". As a matter of fact, BOTALLUS (1565), BINNINGERUS (1673) already observed the manifestations of "hay fever" accompanied by a gasping for breath. BLACKLEY in 1873, also experienced this expiratory difficulty to breathe after inhalation of pollen.

The seasonal tendency of asthma to occur along with the pollinosis symptoms, is well-known today. SCHEPPEGRELL (1922) encountered in the series of 1000 "hay fever" patients 43 % who suffered from asthma during the "hay fever" season.

COCA, WALZER and THOMMEN (1931) stated that pollen asthma develops in the "hay fever" season and does not always terminate at the end of the "hay fever" season, especially after several seasonal recurrences.

RACKEMANN (1931) stressed the fact that pollinosis sufferers and

asthmatics belong to the same family (with eczema in childhood). TUFT (1937) reported that the number of patients who develop asthma has been estimated from 30-60 %. In many patients he admitted "attacks of cough or bronchitis occur instead of a true asthmatic seizure".

FEINBERG (1946) reported 30-40 % of cases of autumnal pollinosis accompanied by asthma and said that the asthmatic symptoms may persist long after the pollen season.

ALBERT (1960) stated that the seasonal recurring asthma due to pollen is a distinct form of pollen sensitivity. ORIE et al. (1961) stated that from 17 "hay fever" sufferers treated for their "hay fever", 11 later on had definite chronic bronchitis. On the other hand, ROWE (1937) studied 1102 patients with bronchial asthma, and encoutered 20 % who had "hay fever" too.

ad b. *Urticaria*

Urticaria associated with pollinosis due to pollen may occur, but is rare.

ad c. *Systemic*

Systemic manifestations associated with pollinosis include such symptoms as: lassitude, insomnia, mental depression and, rarely, some loss of weight. In some patients anorexia may occur but digestive disturbances are uncommon. Sometimes the symptoms (systemic) are due to drugs (antihistaminics).

7. *Family and personal history*

COKE (1933) examined 400 "hay fever" patients and encountered a positive family history for "allergy" in 66.5 %. ROWE (1937) studied 1102 asthmatic patients of whom 218 were pollinosis sufferers too, and found a positive family history for "allergy" in 131 of the 218 pollinosis patients.

HANSEL (1936) stated that there exists a definite family predisposition - and admitted that in about 66 % of adults and 75 % of children there is a family history of "allergy".

DOELEMAN (1957), pointed out the importance of the family and

personal history of diseases linked to the asthmatic constitution - infantile eczema, "hay fever", asthma and bronchitis and encountered a positive history in 80 % in own series. The incidence of constitutional phenomena in the family-members of control, bronchitis and asthma children is shown in table 1.

Table 1

The incidence of constitutional phenomena in the family-members of control, bronchitis and asthma children (Doeleman).

Constitutional phenomena	N: 584 control	N: 1038 bronchitis	N: 2400 slight asthma	N: 210 severe asthma
Absent	80.3 %	43.6 %	49.5 %	47.1 %
Bronchial asthma	11.5 %	48.9 %	37.8 %	37.6 %
Atopic dermatitis	9.8 %	13.9 %	18.9 %	26.2 %
Pollinosis	4.8 %	7.9 %	10.8 %	11.0 %

(From Orie et al., 1961).

8. *Pathological changes*

The nasal pathological changes occurring in the tissues of the pollinosis patients consist essentially of marked oedema of the epithelial and subepithelial tissue with cellular infiltration (eosinophils). The presence of many eosinophils in the secretions as well as in the tissues is characteristic for this allergic ailment.

In the more advanced stages, thickening, hyperplasia and polypoid degeneration of the epithelial layer occur with oedema of the subepithelial layer, proliferation of connective tissue and eosinophilic and mononuclear infiltration. As the result of secondary invaders infection may occur with infiltration of neutrophilic leukocytes and decreasing numbers of eosinophils.

Lastly, there is the formation of polyps.

9. *Laboratory findings*

The characteristic laboratory findings in pollinosis are:

a. positive skin tests;
b. the presence of many eosinophils in the nasal secretion.

ad a. *Skin tests*

In pollinosis, a positive skin test, especially positive scratch tests

is almost conclusive evidence of clinical sensitivity. However, a positive skin test for pollinosis can be regarded as *only* significant when it is correlated with a clinical history of pollinosis. There are also those, especially old people with positive skin tests for pollen without clinical signs of pollinosis during the pollination of the specific pollen. However, when individuals suffer from true pollinosis during the pollination period, it can be said that these subjects will show positive skin reactions to the pollen to which they are sensitized.

ad b. *Eosinophils*

In pollinosis, large numbers of eosinophils are found in the smears of the nasal secretion. In this connection, VOORHORST (1961) found a good quantitative correlation between the quantity of eosinophil cells in the nasal mucus and the degree of blood-eosinophilia during the "hay fever" season. However, according to VOORHORST (1961) an increased number of eosinophilic cells in the blood can be found only in the "hay fever" season. The same holds true for the presence of eosinophils in the nasal mucus, which implies that the eosinophils will be disappeared when the exposure to the (grass) pollen has ceased.

There seems to be a correlation between the severity of the "hay fever" complaints and the degree of eosinophilia and a difference in the presence of eosinophils in treated and untreated "hay fever" sufferers (VOORHORST, 1961), see table 2.

Table 2

Eosinophil cells in nasal mucus from treated and untreated pollinosis patients in summer and winter, according to Voorhorst (1961).

	Eosinophil cells in nasal mucus				
	—	+	++	+++	Total
Summer 1957 (untreated)	1 = 5.3 %	7 = 36.8 %	4 = 21.1 %	7 = 36.8 %	19
Summer 1958 (untreated)	2 = 8.7 %	6 = 26.1 %	10 = 43.5 %	5 = 21.7 %	23
Summer 1958 (treated)	18 = 54.5 %	7 = 21.2 %	6 = 18.2 %	2 = 6.0 %	33
Winter '57/58 (untreated)	28 = 80.0 %	6 = 17.1 %	1 = 2.9 %	0 = 0 %	35

10. *Rhinoscopy*

On rhinoscopic examination, different pictures of the nasal mucosa

may be encountered, e.g. normal, bluish, grayish or red, pale or oedematous. Nasal polyps may also be seen, especially in the "pale mucosa" type.

§ 2. "Vasomotor rhinitis"

1. *Introductory note*

As has been stated in § 1, the nasal symptoms: obstruction, watery discharge and sneezing attacks, are characteristic of pollinosis. These symptoms are the expression of the specific reaction which occurs when a specific agent comes into contact with the nasal mucosa of a sensitized patient. When this contact is seasonal and the agent is pollen the condition is called pollinosis. When these symptoms occur non-seasonally, the nasal condition has been called perennial hay fever, allergic rhinitis, atopic coryza, hyperesthetic rhinitis, perennial rhinitis, nasal catarrh, nasal asthma, paroxysmal rhinorrhea, nervous coryza, nasal neurosis, catarrhal rhinitis, vasomotor rhinitis, etc. — a confusing array of synonyms!

Many investigators have attempted to divide these cases according to the aetiological factors, e.g. inhalants, food, bacteriological factors, disorders of internal secretion and an unbalanced autonomic nervous system. Thus, an attempt has been made to classify the non-seasonal nasal condition into allergic and non-allergic aetiological categories, which will now briefly be considered.

2. *Aetiological factors*

a. *Allergy* (specific, of the immediate type)

The allergic origin of the nasal condition called "vasomotor rhinitis" has first been suggested by LANGLOIS (1906), BILLARD (1910), PERCEPIED (1912), GOODALE (1916), COOKE (1918, 1922), WALKER (1920), TURNBULL (1920), SELFRIDGE (1920), RICH (1922), VAUGHAN (1922), HANSEL (1924) and COAKLEY (1930).

As time went by, many reports by other investigators suggesting allergy as an aetiological factor in "vasomotor rhinitis" followed, e.g. BRAY (1931), RUDOLPH and COHEN (1934), STEVENS (1934), HUBER and HARSH (1934), FORMAN (1934), TUFT (1937), JACKSON and JACKSON (1945), FEINBERG (1946), URBACH and GOTT-

LIEB (1946), HANSEL (1953), HARRIS and SHURE (1957), SHERMAN and KESSLER (1957), CLERICI and TEATINI (1961), VOORHORST (1963), JAMES (1965).

Other forms of allergy [e.g. bacterial allergy (delayed type)] have also been suggested as a cause of the nasal symptoms, e.g. WALKER (1920), GOODALE (1922), GOTTLIEB (1927), KÄMMERER (1928), BRAY (1931), TUFT (1937), URBACH and GOTTLIEB (1946), VAN DISHOECK (1961), HLAVÁČEK (1961).

b. *Non-specific (non-allergic) hyperreactivity*

RACKEMANN (1931) gave an extensive review of "vasomotor rhinitis" and stated that in spite of a careful study of the history, there still remains a considerable group of patients with "vasomotor rhinitis", the cause of whose symptoms could not be identified.

Different reports from the literature support the existence of this non-specific factor in the nasal ailment called "vasomotor rhinitis".

Definition: with non-specific (non-allergic) hyperreactivity is meant an increased susceptibility of the nasal respiratory mucosa to aspecific stimuli, e.g. chemical and physical agents.

"In most instances physical factors probably behave as secondary irritants. In some, physical stimuli, particularly cold and heat, may actually take the role of allergic excitants" (FEINBERG, 1946).

Nasal and bronchial reactions due to physical agents, e.g. cold air, have been reported by DUKE (1924, 1925).

SHERMAN and KESSLER (1957) reported that substances and physical changes which do not induce specific allergic sensitization may precipitate a reaction in the allergic shock organ indistinguishable from the reaction induced by exposure to the specific antigen. HARRIS and SHURE (1957) described "allergic reactions" due to physical agents. These authors advocated the hypothesis that the symptoms from physical agents are due to histamine or an H-like substance which is released directly from the cells on exposure to these agents.

SEEBOHM et al. (1958) stated that a low threshold to physical stimuli is a characteristic of "vasomotor rhinitis".

VAN DISHOECK and VAN LIER (1963) studied this non-specific component by testing the effect of exposure to known amounts of

irritants in normal and certain pathological individuals suffering from "vasomotor rhinitis". These experiments were carried out with ammonia, tobacco, pepper and veratrine. From these experiments it appeared that no reaction could be provoked in normal test persons (students) by insufflation of 1 mg of pepper with 19 mg of rice powder (5 %), while in patients suffering from "vasomotor rhinitis" strong reactions were seen. Inhalation of ammonia had the same effect in this group of patients.

They stated that: "the special nasal condition responsible for heightened susceptibility for irritants does not exclusively belong to non-specific rhinitis vasomotoria, but is linked to both kinds of nasal allergy. Thus in specific allergy, external unspecific irritants may be an additional cause of the attacks apart from the allergen-antibody reaction".

These authors further stated that atopic patients (patients with immediate type skin reactions) suffered more from sneezing attacks than the aspecific "vasomotor rhinitis" group. Furthermore, the patients in both groups who were known to react strongly on pepper stimulation proved also to have a low "sneezing reflex" threshold. They studied 15 "hay fever" patients during summer and winter, and found that these patients proved to be much more sensitive in summer than in winter.

CLERICI and TEATINI (1961) concluded: "we still consider the physical stimulus as an extra-allergic factor, which may possibly favour or condition the allergic rhinitis and which in some instances may even be considered as the cause of the disease".

JAMES (1965) suggested that physical agents (e.g. smoke, fumes or sulphur gas, changes in temperature and humidity) play an important role in "vasomotor rhinitis".

c. *Bacterial factors*

It has never been proved that bacteria or their products are the cause of nasal obstruction, hypersecretion, or sneezing attacks as seen in "vasomotor rhinitis". However, the bacterial factors as the cause of the nasal manifestations have been emphasized by various investigators [e.g. WALKER (1920), GOODALE (1922), GOTTLIEB (1927), KÄMMERER (1928), BRAY (1931), KERN and SCHENK (1933), FEINBERG (1946), URBACH and GOTTLIEB (1946), EGGSTON and WOLFF (1947),

HARRIS and SHURE (1957), PRIGAL (1960), VAN DISHOECK (1961)]. ARIËNS (1964) stated: "infections, too, are a contributive factor in a number of cases of vasomotor rhinopathy. The hypersecretion, the exudate formed and the local inflammation will contribute to the congestion".

JAMES (1965) emphasized the fact that direct action of bacteria and viruses, or their products on the tissue cells constitutes one of the most important predisposing factors.

d. *Endocrine dysfunction*

From the literature, different investigators emphasized endocrine dysfunction as the cause of the nasal manifestations [e.g. SELFRIDGE (1920), HUBERT (1930), HUBER and HARSH (1934), FEINBERG (1946), URBACH and GOTTLIEB (1946), PROETZ (1948), HANSEL (1953), JAMES (1965)].

HUBER and HARSH (1934) analysed cases of "vasomotor rhinitis" and concluded that 70 per cent were allergic, while in the remainder, ". . . non-allergic vasomotor rhinitis group, endocrine dysfunction may be considered as an etiologic possibility". These authors further suggested an underlying endocrine dysfunction because of the exaggeration of the nasal symptoms with the menopause.

FEINBERG (1946) suggested endocrine dysfunction as a possible cause in "vasomotor rhinitis" and stated: "one cannot escape the impression that endocrine function plays a role in hyperesthetic rhinitis, as well as in other allergic conditions".

PROETZ (1948) studied 130 cases of "vasomotor rhinitis" and found improvement of the complaints on treatment with thyroid substance. When the treatment was withdrawn, relapse occurred!

JAMES (1965) also suggested endocrine factors as an aetiological factor in the manifestation of "vasomotor rhinitis" and stated: "hypersecretion is produced by emotional stress and during menstruation and pregnancy".

e. *Autonomic nervous system*

EGGSTON and WOLFF (1947) took care to point out that only about 10 % of all cases of "vasomotor rhinitis" can be proven to

be allergic. "The other cases still remain quite a problem to the physician and patient and the basic mechanism involved is far from understood". These authors further suggested a neurogenic factor in most of these patients.

STEINMANN (1948) investigated 20 patients with Horner's syndrome after traumatic injury to the cervical sympathetic plexus, and found that such injury is followed by a transitory swelling of the nasal mucosa, relapsing at the latest after three years, and then becoming permanent.

HOLMES, GOODELL, WOLF S. and WOLFF H. (1950) stated that during the phase of intense nasal hyperfunction induced by stellate ganglion block, the functionally altered mucous membrane reacts promptly and vigorously to contact with a noxious agent and concluded that cholinergic impulses to the nasal mucous membrane, probably transmitted by the greater superficial petrosal nerve, are responsible for the production of the nasal hyperfunction.

These authors further stated that the impulses mediated by cholinergic fibres in the greater superficial petrosal nerve, are responsible for the production of the pattern of nasal hyperfunction. These observations are in accordance with other investigators [CHOROBSKI and PENFIELD (1932), FENTON and LARSELL (1934), FOWLER (1943), GARDNER et al. (1947), etc.].

STOKSTED and THOMSEN (1953) studied a person with Horner's syndrome and also found a markedly swollen and intensively red nasal mucosa on the affected side, and on the other side a pale-bluish mucosa. VAN DISHOECK and VAN LIER (1963) experimentally changed a normal reaction to 10 mg pepper (by eliminating the stellate ganglion with 1 % novocaine) into a lengthened and violent reaction to 1 mg pepper. These authors concluded that "the cooperation between sympathicus and parasympathicus in the nasal area" is disturbed in those "vasomotor rhinitis" patients who display violent reactions to non-specific stimuli.

GOLDING-WOOD (1963) explained the nasal obstruction, increased secretion and sneezing attacks in "vasomotor rhinitis" as the result of a parasympathetic overactivity. He "cured" 69 (total 74) patients suffering from "vasomotor rhinitis" by means of section of the final cholinergic effector pathway.

More recently ARIËNS (1964) claimed a constitutional hyper-

reactivity of the mucous membrane in the nose to various stimuli as a primary factor in vasomotor rhinopathy. He postulated the possibility that an unbalance in the autonomic regulations is involved.

f. *Drugs*

Certain drugs have been reported to be of causal or additional factors in the nasal manifestations - obstruction, sneezing and hypersecretion. In this connection, the following are of interest.

Reserpine and its derivatives (e.g. guanethidine, bretylium) as nasal obstructing agents [BRODIE and SHORE (1956), CONNOR et al. (1957), KROGSAARD (1958), GOTH (1961), WEDER (1962), MEYLER (1963), MATHOV and MISENTA (1963), ARIËNS (1964), etc.].

KROGSAARD (1958) encountered nasal obstruction in 41 % (total: 75 patients) of his patients on treatment with hypotensive drugs. "The autonomic effects of reserpine may be visualized as being the consequence of decreased sympathetic activity and concomitant parasympathetic prodominance" (GOTH, 1961). He concluded that beside other effects, "nasal stuffiness" may occur, possibly because of decreased sympathetic tone to vessels of the nasal mucosa. In this connection, BRODIE and SHORE (1956) suggested an increased central parasympathetic tone.

ARIËNS (1964) stated, that these drugs act by blocking the release of neurotransmitters at the terminals of the nerve fibres of the sympathetic section of the autonomic nervous system. "About two thirds of hypotensive patients treated with Rauwolfia preparations complain of mild to severe nasal congestion" (CONNOR et al. 1957).

Sympatholytics, e.g. phentolamine and dibenzyline, may cause nasal obstruction [GREEN (1954), KRATZ and CARR (1961), MEYLER (1963) and ARIËNS (1964)].

Other drugs which have been reported are:

Adrenaline - as the result of the so-called rebound congestion, may cause nasal obstruction possibly by means of the beta-sympathomimetic component.

Isoproterenol (beta-receptor) - may cause nasal obstruction by means of the vasodilator action [LEGIER (1958) and ARIËNS (1964)].

Acetylsalicylic acid, quinine and *amidopyrine* have been reported by different investigators, e.g. VALLERY-RADOT and HEIMANN (1930),

DAWSON and NEWMAN (1931), GRIEBEL (1938), BALLENGER (1944), SHERMAN (1947) and JAMES (1965).

Increased secretion may be caused by the iodides used as expectorants [ARIËNS (1964) and JAMES (1965)].

3. *Symptomatology*

The nasal symptoms are characterized by obstruction, hypersecretion, itching and sneezing. Anyone or a combination of these symptoms may dominate the clinical picture. "The patient complains bitterly of chronic nasal obstruction with resulting dry throat and discomfort in his head.. in the typical case, a paroxysm of sneezing is likely to occur when the patient first gets out of bed.." (RACKEMANN, 1931). On the whole the symptoms are often the same as in pollinosis; however, there are some points which may help us to distinguish between these conditions (see table 9).

4. *Influence of sex and age*

"Vasomotor rhinitis is a disease of young people... Women are much more commonly affected than men - 73 per cent as against 27 per cent" (RACKEMANN, 1931). This author studied 257 cases of "vasomotor rhinitis", and gave the following figures:

Table 3

The age of onset of "vasomotor rhinitis" (according to Rackemann, 1931).

Decades of life:	1	2	3	4	≧ 4	Total No.
No. of "vasomotor rhinitis" patients	36	64	83	47	27	257

BRAY (1931) stated that "allergic rhinitis" (perennial type), like "hay fever", occurs most commonly from 15-40 years of age, but is twice as frequent in females as in males (see fig. 3).

HUBER and HARSH (1934) reported that females constituted 80 % of their material, while URBACH (1941) claimed 54 %.

VAN DISHOECK (1961) stated that "... non-atopic nasal allergy was seen about twice as often as atopic nasal allergy, with a high percentage of young women among the non-atopic group".

URBACH and GOTTLIEB (1946) encountered 39.7 % males and 36.0 % females. CLERICI and TEATINI (1961) stated that extra-allergic "vasomotor rhinitis" is most commonly seen between the ages of 20 and 40 years.

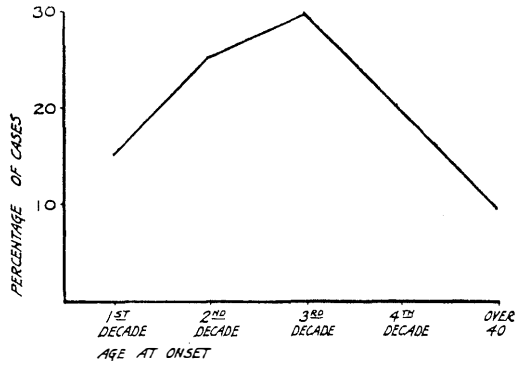


Fig. 3
The age-incidence of "vasomotor rhinitis" bij decades.
(From Bray, 1931).

GOLDING-WOOD (1963) reported that "cholinergic cases" ("vasomotor rhinitis") mostly occur between 20-40 years of age.

5. *Associated manifestations*

a. Asthma
b. Headache
c. Systemic

Ad a. *Asthma*

RACKEMANN (1931) stated: "just as one third of all our patients with hay fever, wheeze and therefore have asthma during the hay fever so with vasomotor rhinitis, we find that 131 of our total 356 patients (36.8 per cent) have asthma at the same time".

BRAY (1931) suggested asthma in nearly two out of every three of these patients. TUFT (1937) found "slight cough, heaviness or constriction in the chest" and concluded: "it is to be remembered, of course, that the attack of allergic coryza and asthma may be produced by the same cause".

FEINBERG (1946) reported that at least a third of the cases of "vasomotor rhinitis" is associated with various degrees of asthmatic symptoms. ROWE (1937) concluded that many of these patients suffer from concomitant cough or asthma. SHERMAN and KESSLER (1957)

reported that the incidence of asthma "in untreated cases (children)" has been estimated to be 30-50 %. These authors emphasized this co-existence of "rhinitis" and "asthma" and concluded that the association of cough with the "rhinitis", or recurrent attacks of bronchitis, forms a demand for intensive treatment.

CLERICI and TEATINI (1961) also reported the frequent association of "vasomotor rhinitis" (extra-allergic) with what they called - spastic tracheitis or bronchial asthma.

The co-existence of "vasomotor rhinitis" and asthma (bronchitis) according to various authors is given in table 4.

Table 4

The co-existence of "vasomotor rhinitis" and asthma (bronchitis) in percentage according to various authors.

BALYEAT (1929)	36 %
RACKEMANN (1931)	36.8 %
WINKENWERDER and GAY (1937)	25 %
WOODWARD and SWINEFORD (1941)	59 %
HALD (1943)	14 %
HANSEL (1953) - Adults	25 %
- Children	69 %
SCHNYDER (1960)	15.3 %
VAN LIER (1960)	15%**
	42 %*
WEDER (1962)	15 %

* Asthma in atopic "vasomotor rhinitis"
** Asthma in non-atopic "vasomotor rhinitis"

Other investigators, studied cases of bronchial asthma and encountered many instances in which both - bronchial asthma and "vasomotor rhinitis" occurred simultaneously, e.g. URBACH and GOTTLIEB (1946) encountered 38 % (total: 379 asthmatics) and reported that "... almost half of these cases, the rhinopathy had appeared simultaneously with the asthma, in these, the two conditions were invariably found to be due to the same agent - allergen or pathergen". KREUKNIET (1959) encountered allergic (32.3 %) and non-allergic (12.9 %) nasal conditions in an asthmatic group of patients.

VAN DISHOECK (1954) pointed out that the combination of "vasomotor rhinitis" and asthma is very frequently seen and, as a matter of fact, he suggested the same mechanism and cause for both conditions and concluded that the only difference is the shock organ.

ad. b. *Headache*

Patients suffering from "vasomotor rhinitis", especially those with chronic nasal obstruction, complain of headache [SLUDER (1919), POLLOCK (1919), PALMER (1935), TUFT (1937), BURNHAM (1937), HOFER (1963), DE WIT (1965), SLOME (1965), etc.].

As the result of swelling of the nasal mucosa, the extreme sensitive sinus ostia are closed off with retention of mucus.

ad c. *Systemic*

In "vasomotor rhinitis" patients, general symptoms are not usually present. When the attacks are severe, however, "fatique, irritability, lack of concentration and anorexia may occur" (JAMES, 1965). In persistent nasal obstruction, the throat is likely to be dry and irritated as the result of mouth breathing.

6. *Clinical course*

The clinical course and symptomatology of patients with the nasal manifestation of "vasomotor rhinitis", are subjected to variations. The frequency of occurrence of nasal obstruction, sneezing and hypersecretion varies from patient to patient. In some patients the attacks occur at intervals. Some patients get up in the morning with an obstructed nose, while others are seized with sneezing attacks after awakening, and on getting out of bed. In others the nasal symptoms - especially nasal obstruction - may be more or less constant instead of paroxysmal. Furthermore, the degree of nasal obstruction may vary at different periods of the day, accompanied with very slight increased secretion and/or very few or no sneezing fits. In others again, persistent nasal obstruction is the main symptom during the whole day.

The characteristics, intensity and duration of the nasal symptoms may be influenced by different stimuli, e.g. the slightest exposure to smoke, changes in temperature and cold air, may provoke nasal obstruction, sneezing and/or hypersecretion. The onset of the symptoms may be sudden or gradual. In some patients the symptoms may persist in a mild form for months or years and eventually disappear, while in others the symptoms may increase in severity and duration resulting in infection and pathological changes. The incidence and the age of onset have already been mentioned.

7. *Family and personal history*

RACKEMANN (1931) reported that the evidence of an allergic background in his series as a whole, was not striking. He obtained a positive family history of "allergy" in 25 %. A history of eczema or urticaria was obtained in 7 %. BRAY (1931) emphasized the fact that a personal or family history of "other allergy" is not so frequently obtained as in the true "hay fever" patients. HANSEL (1936) studied 220 adults with the nasal manifestations of "allergy" and found 135 (61.4 %) who gave a positive family history for 'allergy' - 79 were males and 141 females. In the males the family history was positive in 68.35 %, and in the females in 57.05 % (see table 5 and 6).

Table 5

Family history of allergy - males (adult).

	Total	Per cent	Nasal A.	Asthma	Headache	Hay fever	Eczema	Urt.	G. I.	Total
Maternal	29	53.7	7	12	9	3	4	6	1	42
Paternal	10	18.5	3	3	2	2	0	1	4	15
Bilateral	4	7.4	3	2	2	2	0	1	0	10
Bro. only	2	3.7	1	0	1	0	0	0	0	2
Sis. only	7	13.0	3	0	1	0	0	3	0	7
Child. only	2	3.7	2	0	0	0	0	0	0	2
Pos. family history	54	(68.35 %)	19	17	15	7	4	11	5	78
Neg. family history	25	(31.65 %)								
Total	79									
Additional Family History of Allergy										
Brother	9		3	0	2	2	0	1	1	9
Sister	6		2	0	1	0	2	1	0	6
Total	15		5	0	3	2	2	2	1	15
Total manifestations			24	17	18	9	6	13	6	93

(From Hansel, 1936).

Table 6

Family history of allergy - females (adult).

	Total	Per cent	Nasal A.	Asthma	Headache	Hay fever	Eczema	Urt.	G. I.	Total
Maternal	39	48.2	13	20	18	7	2	0	2	62
Paternal	7	8.6	5	8	2	0	0	0	1	16
Bilateral	12	14.8	6	7	3	5	1	1	0	23
Bro. only	6	7.4	1	1	1	2	1	0	0	6
Sis. only	11	13.6	2	1	0	3	4	1	0	11
Child. only	6	7.4	4	1	0	0	1	0	0	6
Pos. family history	81	(57.5 %)	31	38	24	17	9	2	3	124
Neg. family history	60	(42.5 %)								
Total	141									
Additional Family History of Allergy										
Brother	10		5	1	2	1	4	0	0	13
Sister	11		2	0	6	3	2	0	0	13
Son	7		2	0	0	3	2	1	0	8
Daughter	5		1	0	0	0	0	3	0	4
Total	33		10	1	8	7	8	4	0	38
Total manifestations			41	39	32	24	17	6	3	162

(From Hansel, 1936).

HANSEL (1936) also studied a group of 200 children with the nasal manifestations of allergy and gave the following figures (table 7).

Table 7

Inheritance and incidence of manifestations in family history (children).

	Total	Per cent	Asthma	Hay fever	Nasal A.	Eczema	Urt.	Headache	G. I.	Angio.	Total
Male											
Maternal	42	54.5	22	11	8	6	10	9	1	2	69
Paternal	16	20.8	8	7	3	1	3	1	0	0	23
Bilateral	16	20.8	19	5	3	5	6	4	0	1	43
Sis. only	1	1.3	0	0	1	0	0	0	0	0	1
Bro. only	2	2.6	0	0	1	0	1	0	0	0	2
Pos. family history	77	(68.1 %)	49	23	16	12	20	14	1	3	138
Neg. family history	36	(31.9 %)									
Total	113										
Female											
Maternal	31	46.3	19	6	9	6	4	8	3	0	55
Paternal	16	23.9	10	4	1	3	1	2	0	0	21
Bilateral	13	19.4	15	7	6	7	2	2	0	0	39
Sis. only	2	3.0	1	0	0	1	0	0	0	0	2
Bro. only	5	7.4	1	0	0	3	1	0	0	0	5
Pos. family history	67	(77.0 %)	46	17	16	20	8	12	3	0	122
Neg. family history	20	(23.0 %)									
Total	87										

(From Hansel, 1936).

Regarding the personal and family history, TUFT (1937) stated: ".... a history of this is not obtained as often as in asthma or hay fever. Conversely, however, a positive family history makes it more likely that the condition is due to an allergic factor". The manner, TUFT said, in which heredity influences the onset, is the same in this condition as in asthma. He suggested a special predisposition to acquire this condition, because he admitted that allergens (allergy), although important, are not the only essential factor.

Positive family histories for nasal obstruction, sneezing and hypersecretion have also been reported by investigators who primarily studied affections of the lower respiratory tract, e.g. ROWE (1937) encountered a positive history for nasal obstruction in 35 % (total: 1102 patients); VAN DER WAL (1964) found 29.3 % (total: 150 subjects).

8. *Pathological changes*

The pathological changes occurring in the nasal tissues (and accessory sinuses) of patients with "vasomotor rhinitis" are generally the same as those in "hay fever" [BRAY (1931), TUFT (1937) and HANSEL 1953)]. During the symptom-free periods, the oedema may decrease so that the nasal mucosa appears normal. After an exacerbation of the nasal symptoms, a gray-pallor may be seen, the degree of which is dependent upon the extent of the oedema. If the oedema persists for a longer time, the elastic tissue and muscular walls of the vessels and stroma may be weakened resulting in a chronic swollen condition. The chronic swollen condition of the nasal mucosa, blocking the normal air passage and sinus antra will promote secondary infection with the prominent characteristic of polymorphonuclear neutrophile cellular picture. Lastly polyps are formed, mainly, as the result of the vascular changes in the nasal mucosa (EGGSTON and WOLFF).

9. *Laboratory findings*

a. *Eosinophils*

As has been stated, a characteristic laboratory finding in pollinosis is the presence of many eosinophils in the nasal secretions (and tis-

sue). In "vasomotor rhinitis", eosinophils are also found in patients sensitized to allergens; however, when no allergy can be found, the eosinophilic cells are usually scarce or absent [EGGSTON and WOLFF (1947), ALBERT (1960) and VOORHORST (1961)]. In this connection, VOORHORST (1961) stated: "the atopic form is closely connected with the reactions of the eosinophil cells", and ".... in the patients with non-atopic vasomotor rhinitis and asthma a certain degree of reaction of the eosinophil system may exist, but this is usually much weaker than in the atopic cases".

b. *Skin tests*

In "vasomotor rhinitis" (in contradistinction to pollinosis) a positive history of nasal obstruction, hypersecretion and sneezing does not necessarily indicate positive skin reactions to allergens. Furthermore, in "vasomotor rhinitis", a positive skin reaction to a certain allergen(s) does not necessarily indicate clinical sensitivity.

The diagnosis whether a patient suffers from allergic or non-allergic "vasomotor rhinitis", is usually based in the literature on the presence or absence of skin reactions to allergens, as FEINBERG (1946) stated: "although a large proportion of patients with hyperesthetic rhinitis show no reactions to skin tests, skin tests still constitute the most important diagnostic procedure in this condition".

However, one cannot consider allergens and skin tests in relation to nasal manifestations without mentioning the importance of identification of clinical sensitivity by means of nasal provocation tests, because ".... the skin, it seems, is the allergic bookkeeper of the body, recording past, present and perhaps future sensitivity" (PRIGAL, 1960).

Data gathered in table 8 show the percentage of negative skin tests in "vasomotor rhinitis".

Table 8

Negative skin tests in "vasomotor rhinitis".

RACKEMANN (1931)	42 %
HUBER and HARSCH (1934)	30 %
BAUM (1934, 1935)	72.7 %
KUHN and LINTON (1942)	80.6 %
URBACH and GOTTLIEB (1946)	81.0 %
EGGSTON and WOLFF (1947)	± 90.0 %
WEBER (1962)	60 %

10. *Rhinoscopy*

Different pictures of the nasal mucosa may be encountered, e.g. normal, bluish, grayish or red, oedematous or pale. Nasal polyps may also be seen.

§ 3. Differential Diagnosis

Table 9

Features	Pollinosis	"Vasomotor rhinitis" Allergic	Non-allergic
Nasal obstruction	Moderate to marked	Moderate to marked	Marked
Nasal hypersecretion	Colourless, watery profuse	Watery, colourless, profuse	Watery - scant to moderate
Sneezing	Marked	Moderate	Moderate
History of "allergy"	Present	May be present	Usually absent
Eye symptoms	Present	May be present	Usually absent
Duration of symptoms	Confined to pollen season	Hours to few days (when in presence of offending allergen)	Hours, months and years
Nasal eosinophilia	Present	Present	Usually absent
Positive skin tests	Always present	Present	Absent or coincidental
Family history of "allergy"	Present	Present	Usually absent
Complicating asthma	May be present after several seasons of nasal symptoms	May be present	May be present

[Some data obtained from Albert (1960)].

Chapter III

ASSESSMENT OF THE REACTIVITY OF THE NASAL RESPIRATORY MUCOSA

§ 1. Introduction

As has been stated in the introduction the purpose of this investigation is to demonstrate reactivity patterns of the nasal respiratory mucosa in patients with nasal and chest complaints and in normal populations (which will be discussed later on). It has been postulated that the reactivity of the nasal mucosa can be described in terms of the relationship between a stimulus and the effect provoked by this stimulus.

One can speak of a difference in the reactivity of one person in respect to another, when quantitatively different reactions are caused by the same stimulus.

In order to have a working notion: "reactivity of the nasal respiratory mucosa", it is necessary to describe more explicitly the following terms:

a. the stimulus and its effects;
b. the measurement of the effect;
c. the relationship between the stimulus and the effect.

a. *The stimulus and its effects*

In earlier clinical investigations, histamine has been used to provoke reactions in the nasal mucosa (VAN DISHOECK, INGELSTEDT and IVSTAM, MÉLON and LECOMTE, MÉLON, etc.). Reactions such as increased secretion, sneezing and nasal obstruction, which closely resemble the complaints in patients with rhinopathy, have been observed.

In view of these observations, histamine has been used in this investigation as the "model agent". The procedure of histamine application will be described in § 3.

The following generally known pharmacological effects of histamine can be considered.

1. *Contraction of smooth muscle* [e.g. DALE and LAIDLAW (1910, 1911 & 1918), GUGGENHEIM and LÖFFLER (1916), MAUTNER and PICK (1915, 1922 & 1928), ROSE (1947), ROCHA E SILVA (1955), HALPERN (1960), GROLLMAN (1960), GOTH (1961), SLOME (1965)].
2. *Dilatation of capillaries* [e.g. DALE and RICHARDS (1918), HOOKER (1920), CARRIER (1922), RAMSDELL (1928), LEWIS (1924, 1926 & 1927), ROSE (1947), ROCHA E SILVA (1955), HALPERN (1960), GROLLMAN (1960), GOTH (1961), SLOME (1965), SPECTOR and WILLOUGHBY (1965)].
3. *Contraction of small arteries (arterioles) and veins (venules)* [e.g. DALE and RICHARDS (1918), DIXON and HOYLE (1930), ROCHA E SILVA (1955), HALPERN (1960), ZWEIFACH (1961), GOTH (1961)].
4. *Increased capillary permeability* [e.g. DALE and RICHARDS (1918), CARRIER (1922), MCCARRELL and DRINKER (1941), ROSE (1947), ROCHA E SILVA (1955), HALPERN (1960), GOTH (1961), SLOME (1965)].
5. *Increased glandular secretion* [e.g. ROSE (1947), GROLLMAN (1960), HALPERN (1960), GOTH (1961), SLOME (1965)].

Despite the substantial array of information regarding histamine, it is not yet fully known which of these pharmacological properties can be regarded as the most important on the nasal mucosa in man - a lack of unanimity regarding the influence of these properties of histamine on the reaction of the nasal mucosa still prevails. Moreover, some of these actions are dependent on each other [e.g. increased permeability as the result of capillary dilatation, HALPERN (1960)]. Attention will be paid to this problem in chapters V and VIII.

b. *The measurement of the effect*

One of the problems is, how to measure the changes of the nasal respiratory mucosa induced by a stimulant. In the ideal case, one should measure the changes of the blood vessels, but "measurements of vascular reactions in the nasal mucosa were often unsatisfactory"

(DRETTNER, 1961). DRETTNER further stressed the fact that instruments used intranasally, caused swelling, nasal discharge or rise of temperature in the nasal mucous membrane. DAVIS and HERTZMAN (1957) reported a method for photoelectric plethysmography of the septal mucosa. Changes in the amplitude of the volume pulses and in blood content were recorded, but DRETTNER said that the apparatus these investigators used was too heavy and difficult to manoeuvre.

Methods for measuring the temperature of the nasal mucosa have also been reported [MUDD, GOLDMAN and GRANT (1921), RALSTON and KERR (1945), RICHTNER (1943), DRETTNER (1961), etc.]. However, the temperature of the nasal mucosa is dependent on many factors, e.g. arterial blood supply, temperature and humidity of the ambient air. Cooling of the feet, local skin cooling and cooling of the respiratory air are accompanied by a reduction of the nasal temperature (DRETTNER, 1961). These methods have a disadvantage — unpleasant intranasal manipulation. Furthermore, these methods seem unsuitable for investigation into the reactivity of the nasal mucosa on a large scale.

The suggestion was made, that changes of the nasal mucosa (either a decongestion or congestion and hypersecretion) should cause changes in the nasal passage, and that these can be used as a parameter for the assessment of the reactivity of the nasal mucosa.

The problem involved in measuring the nasal passage will be discussed in § 2. The method used in this study is given in § 3.

c. *The relationship between the stimulus and the effect*

The problem of estimating the "reactivity of the nasal respiratory mucosa" from the relationship between a certain dose (of histamine) and the changes in the nasal passage (response) will be discussed in § 4.

The remaining paragraphs in this chapter will deal particularly with technical details which may influence this reactivity. These details can be summarized more efficiently when the method used has been reported (§ 5 and following paragraphs).

§ 2. Measurement of the nasal passage

For the assessment of the nasal passage, many methods have been described in the literature - mainly based on pressure variations in the nasopharynx. The measurements of pressure variations were made either in the nasopharynx or in the nares. Detailed accounts of these methods have been published by various investigators [e.g. UDDSTRÖMER (1940), STOKSTED (1956), SEMERÁK (1958), MALCOMSON (1959) MÉLON (1964), SLOME (1965)]. Therefore, only the principles will be mentioned for a number of methods used [e.g. MCLAURIN et al. (1960), SEEBOHM and HAMILTON (1958), COTTLE (1958, 1963), SOLOMON et al. (1965)].

According to the literature, these methods can be classified as indirect and direct.

A. *Indirect methods*

1. *Non-hygrometric method**

a. *Constriction sound test* [BRUCK (1901), ZARNIKO (1910)].

During forced respiration through one normal nasal chamber, an "f"-like sound is heard, but when the chamber is partially obstructed, a Soundlike Scots "ch" is heard.

b. *Humming test* (SPIESS, 1902)

If one hums ("m"-like sound) with the mouth closed and then alternately closes the right and left nostril, no change in the pitch of the tone occurs when the passage of both nasal chambers is similar. In unilateral nasal obstruction with the non-obstructed side closed, a sinking of a semitone or more, or at any rate a change of timbre occurs. In such a way a comparison can be made of the two nasal chambers in the same patient but no standardised sound exists for comparison with other individuals.

c. *Rosenthal's test* (1904)

The person examined breathes 20 times in succession through both chambers with the mouth closed. This is repeated with first one nostril closed and then the other. Soon, there will be irregular breathing, dyspnoea and a more rapid pulse, when the side through which the patient breathes is obstructed.

* Some data obtained from Uddströmer.

2. *Hygrometric method*

By means of the non-hygrometric methods, the nasal passage is more or less subjectively evaluated, but by the introduction of the hygrometric method a kind of objective registration was introduced by ZWAARDEMAKER in 1889.

This method is based on the principle that the water vapour in the expired air is condensed on a cold mirror held under the nostrils producing the so-called "breathing spots". From the size and duration of the respiratory spots on the mirror he drew his conclusions concerning the size of the air passage. This method has further been modified and improved by others [SANDMANN (1893), GLATZEL (1901), COURTADE (1902), ESCAT (1908), FOY (1910), HELLMAN (1926)]. These methods, however, afford merely a gross assessment of the nasal passage.

When applying these indirect rhinometrical methods for the assessment of the nasal passage, it should be considered that:

a) in the case of the non-hygrometric methods, a certain tendency of the alae nasi to collapse may occur when only the one side is tested, while in bilateral respiration such a tendency does not exist;
b) in the case of hygrometric methods, the objectively noted "breathing spots" are related to the hygrometric state of the environmental air and to the respiratory volume, which implies that comparison between different individuals cannot be obtained.

B. *Direct methods*

These methods can be divided into anterior and posterior rhino-(mano)metry.

Anterior rhino(mano)metry

This measurement is performed by connecting the right and left nostril alternatively to a liquid manometer by means of an olive and connecting tubing.

The pharynx-nostril pressure gradient ($\triangle P$) is determined during

respiration through the open non-connected side of the nose, with the mouth closed.

In anterior rhino(mano)metry, the assumption is made that the pressure variations in the nasopharynx equal the pressure variations in the nares. Moreover, it is assumed that the pressure variations are registered accurately by the manometer, but this only holds true when the diameter of the manometer tube is small in respect to the volume in the nasal chamber. When the diameter is large this assumption no longer holds true. The quantity of air transferred is important for the reliability of the measured value. STOKSTED therefore replaced the liquid manometer by a recording diaphragm-type manometer capable of recording the pressure variations during respiration. False information may be obtained on the manometer (no movements or too small movements) when the nasal chamber is totally or partially blocked, since the nasal chamber acts as a pressure cannula (UDDSTRÖMER). Although anterior rhino(mano)metry is simple to manage and can give good preliminary results, there is a restriction in the applicability of this method. Anterior rhino-(mano)metry as an isolated method is uncertain when the flow is not controlled (DRETTNER, STOKSTED).

Anterior rhino(mano)metry, introduced by COURTADE in 1902, was used by various investigators, e.g. TATUM (1923), DUFTON and BEDFORD (1933), HILL (1933), WINSLOW et al. (1934), UDDSTRÖMER (1940), VAN DISHOECK (1942), STOKSTED (1956). It was also used in combination with flow measurements in the non-connected side of the nose, e.g. LEHMANN (1939), SEMERÁK (1958), COTTLE (1958, 1963). In olive connection, as used here, the alae nasi and the ostium internum are put out of action, the cross-sectional area is diminished, and the vestibulum is deformed at the open non-connected side of the nose - all factors which may influence the pressure measurements.

Against the background of the disadvantages of olive connection, a nasal mask has been developed. UDDSTRÖMER (1940) devised a special face mask divided into an oral and a nasal compartment. Each compartment is connected to a gas-meter measuring the total air passing through the mouth and the nose during rest and exercise. Another mask, a so-called "nasal oxygen mask", has been introduced by MCLAURIN et al. (1960).

The advantage of anterior rhino(mano)metry lies in the simple applicability of the method, while the disadvantages are:

a. the principle of the method, viz. the assumption that the chamber including the nasal passage from pharynx to nostril acts as one (pressure) system, cannot always be maintained, because of disturbing factors (e.g. olive connection, total or partial obstruction, polyps, spurs, crests);
b. flow measurements have to be taken (artificially), which implies a co-operation of the patient (e.g. closure of the glottis, apneic periods).

Posterior rhino(mano)metry

In posterior rhino(mano)metry, pressure measurements are made directly in the nasopharynx (or oropharynx). It was introduced by SPIESS (1900): a glass cannula or catheter connected with a manometer is introduced via the mouth behind the soft palate. During respiration through the nose with the mouth closed, the pressure variations in the mesopharynx are registered. The pressure variations in the mesopharynx are accepted as the pressure in the nasopharynx. This form of rhinometry has been used by various investigators, e.g. BEYNE (1926), WORMS et al. (1928), ASCHAN, DRETTNER and RONGE (1958), SEMERÁK (1958).

SEEBOHM and HAMILTON (1958) measured the pharynx-nostril pressure gradient under controlled flow conditions. Oxygen is administered only during voluntary apneic periods. With this method the collaboration of the patient is necessary and errors are possible, due to incomplete closure of the glottis, or by movement of the pharynx during voluntary apnoea.

SOLOMON et al. (1965) measured the pharynx-nostril pressure gradient simultaneously with the flow through the nose during respiration by means of a pneumotachograph. These investigators measured the pressure in the oropharynx. The problem involved in this method is that the uvula and pharyngeal muscles must remain relaxed during the time of the experiment. Several minutes may be required to obtain relaxation of the uvula and pharyngeal muscles. STOKSTED (1956) said that application of a tube behind the soft palate, introduced via the mouth, will often cause unpleasant sensations to the

patient and unintended contractions and reflexes in the soft palate limiting the applicability of the method.

Volumetric measurements

Assessment of the nasal passage based on volumetric measurements has been used to a lesser extent [e.g. MENDEL (1897), TSCHALUSSOW (1913), SCHEIDELER (1939), JACKSON (1942), KOCH and KAPLAN (1966)]. In this form of measurement the collaboration of the patient is also needed (e.g. the patient has to hold his breath).

Summarizing, it can be said that posterior rhino(mano)metry is preferable to anterior rhino(mano)metry for the assessment of the nasal passage. Simultaneous pressure and volume measurements have to be taken.

Of all the different modifications, the direct measurement of the pressure in the nasopharyngeal cavity is considered best.

MALAN in 1928 had already introduced a soft catheter via one of the nasal chambers directly into the nasopharynx, for the assessment of the nasal passage. There is no need for active collaboration of the patient by using spontaneous respiration.

On these principles the method used in this study has been devised and will be described in the next paragraph.

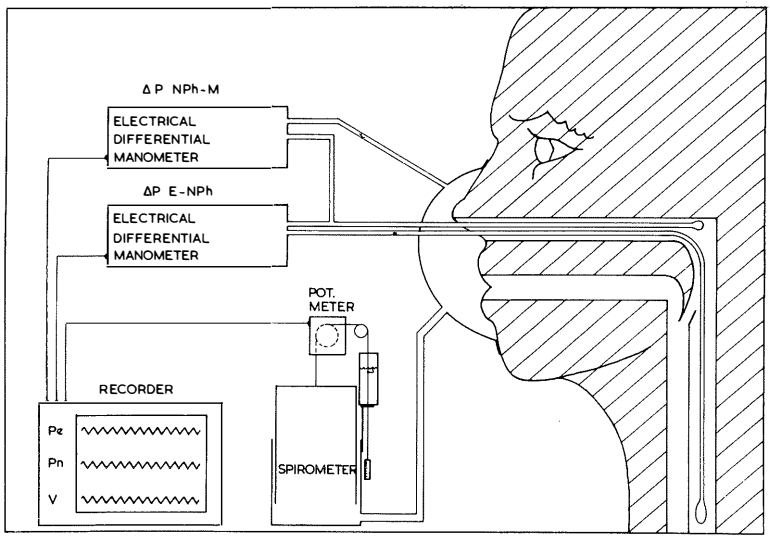
§ 3. Own method of determination of the reaction pattern of the nasal respiratory mucosa to histamine provocation

The method for the assessment of the nasal passage consists of the simultaneous registration of the pharynx-nostril pressure gradient and tidal volume through one nasal chamber during normal respiration. A change in the nasal respiratory mucosa, viz. a swelling of the mucosa, results in a change in the nasal passage which can be recorded quantitatively by measuring the changes in the pharynx-nostril pressure gradient and respiratory volume through the nose.

Furthermore, a special device has been added for measuring changes in the oesophageal pharynx pressure gradient. The method is schematically shown in fig. 4.

Fig. 4

Schematic representation of the method for the assessment of the nasal passage.



Pe : Oesophageal pharynx pressure gradient
Pn : Pharynx-nostril pressure gradient
V : Tidal volume
△P : Pressure difference
NPh-M : Nasopharynx-mouth
E-NPh : Oesophageal-mouth
Pot.meter : Potentiometer.

Equipment used

Four-channel recorder[1]), spirometer[2]), two electrical differential pressure transducers[3]), potentiometer, face mask covering mouth and nose, small balloon (length: 1½ cm, diameter: 5 mm) with polyethylene tubing for measuring the pharynx-nostril pressure gradient, small balloon (length: 14 cm, diameter: 6 mm) with polyethylene tubing for measuring the oesophageal pharynx pressure gradient, and a water manometer.

Description of the procedure

After the shape of the external nose is noted, and anterior and

[1]) Schwarzer EE8, Laméris Instrumenten NV, Biltstraat 149, Utrecht;
[2]) Lode-Spirograaf D53, Instrumenten Lode NV, Oosterstraat 38, Groningen;
[3]) Godart NV, Soestdijkseweg 13Z, De Bilt.

posterior rhinoscopy has been performed, a specially designed balloon is introduced into the nasopharynx via one nostril under rhinoscopic guidance. By means of a polyethylene tubing the balloon is connected to an electrical differential pressure transducer, the output of which is transmitted to a four-channel recorder. After the balloon is filled with 0.5 ml of air via the tube, the exact position of the balloon is checked by means of posterior rhinoscopy to assure that the other side of the nasal passage remains completely free. By means of a face mask, covering the nose and mouth, the respiratory volume through one nostril is registered on a spirometer. Care is taken that the application of the mask to the face is airtight and that the one chamber, through which the balloon is introduced, is completely closed with a cotton wad. Furthermore, care is taken to avoid contact between the mask and the alae nasi. The movements of the spirometer bell are electrically transformed by means of a potentiometer and registered on the four-channel recorder.

While the patient breathes through the nasal chamber connected with the spirometer, simultaneous registration of the pharynx-nostril pressure gradient and tidal volume is obtained.

The pressure readings on the recorder are gauged by means of a water manometer, while the volume readings are gauged by means of the spirometer, after which the initial values are registered. Distilled water is then applied to the nasal mucosa in order to obtain a placebo reading and thereafter, in the case of histamine provocation, mounting concentrations of histamine diphosphate (salt) are applied, viz.: 1/32 (2^{-5}), 1/16, 1/8, 1/4, 1/2, 1, 2, 4, 8, 16 and 32 (2^{5}) mg/ml. The exponential value of 2, e.g. a positive reaction to 1/32 mg/ml of histamine (2^{-5}) will be denoted by -5, to 16 mg/ml (2^{4}) as +4, etc. [2 mg/ml histamine diphosphate (salt) equals 0.7 mg/ml histamine (base)*]. The histamine diphosphate is dissolved in distilled water and applied by means of a wad of cotton on a nasal probe, placed in the nasal chamber for 15 sec.. After the application of each concentration, the pharynx-nostril pressure gradient and tidal volume are registered during normal respiration for $1^{1}/_{2}$ min.. The respiratory frequencies are noted. After a positive reaction of the nasal mucosa (viz. partial or total obstruction), decongestion of the

*) = 0.0065 Mol/lit.

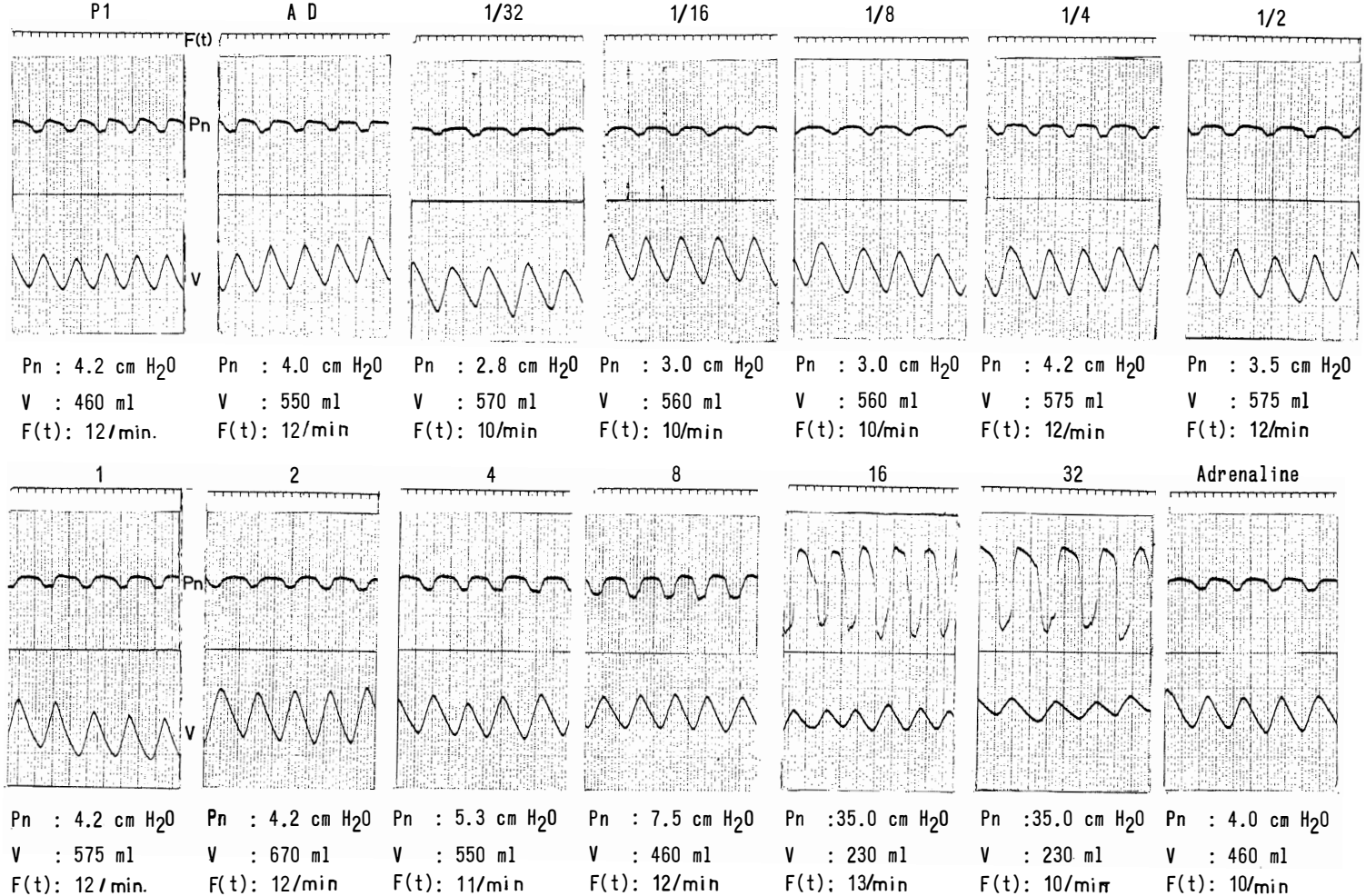


Fig. 5

Examples of curves obtained, showing the mean values for the pharynx-nostril pressure gradient (Pn), tidal volume (V) and respiratory frequencies [F(t)] during one minute, after the application of distilled water (AD), mounting concentrations of histamine, and adrenaline on the nasal

mucosa is obtained by means of application of adrenaline, 1 : 1000.

An example of a curve obtained over a 1 min. period, after application of distilled water and mounting concentrations of histamine, is given in fig. 5. The mean pressure and volume values are taken as parameters for the nasal passage.

The initial reading of the $\triangle P$ obtained in a random sample (see chapter IV) (n = 113) of a normal population has a mean value of 2 ± 1.8 (95 % confidence interval) cmH_2O.

It must be stressed that the ultimate purpose is to demonstrate, quantitatively, changes in the nasal passage and not primarily to perform nasal respiratory function tests.

Difficulties met with during the efforts to achieve a good assessment, of the nasal passage, will be discussed successively. These are:

(1) the criteria necessary to define the reactivity of the nasal mucosa to different stimuli on the basis of changes in the nasal passage (see § 4);
(2) the possible sources of error:
   (a) mechanical irritation and (b) the solvent (see § 5);
(3) topical application vs. nebulization (see § 6);
(4) the influence of the nasal anatomical structure in the assessment of the reactivity of the nasal respiratory mucosa (see § 7);
(5) the reproducibility of the results obtained with the technique described (see § 8);
(6) the possible complications (see § 9).

§ 4. Criteria of the reactivity of the nasal respiratory mucosa to histamine

The reactivity of the nasal respiratory mucosa can be derived from the relationship between the stimulus and the effect obtained. Essential in this connection is the analysis of the dose-response curve, the form and position of which provide the data necessary for the derivation of the criteria for the reactivity of the nasal mucosa.

A dose-response curve can be constructed by taking mean values of the pressures and volumes measured over a 60 sec. period* after

* In the case of total nasal obstruction the measurements are taken during shorter periods (about 12 sec.) for 60 sec.

application of mounting concentrations of histamine. An example of such a curve is given in fig. 6.

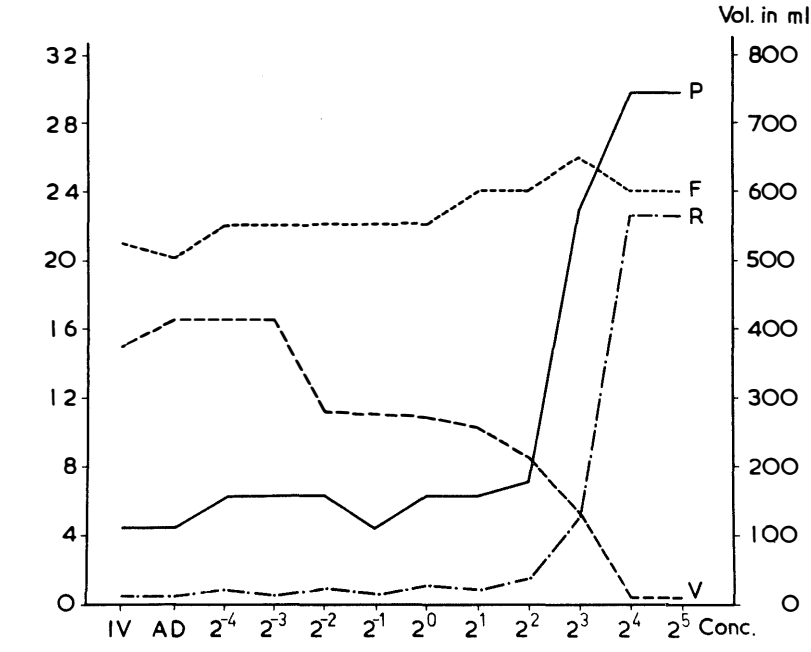


Fig. 6

Dose-response curve of histamine application to the nasal mucosa.

AD: Distilled water
P: Pharynx-nostril pressure gradient in cmH_2O
F: Respiratory frequency per minute
"R": P/V. F(t) in cmH_2O/lit.
IV: Initial value of P, F, "R" and V
V: Tidal volume

Pressure (P): this line shows a minimum plateau base-line after the provocation with 2^{-4}, 2^{-3}, 2^{-2}, 2^{0}, 2^{1}, 2^{2} mg/ml histamine, and after the application of 2^{4}, 2^{5} mg/ml histamine a maximum plateau is reached.

Volume (V): here the opposite picture can be seen — a maximum

plateau after the application of the lower histamine concentrations, and a minimum plateau after the reaction of the nasal mucosa.
Frequency (F): the frequency of respiration remains practically the same after the application of the different concentrations of histamine.
Pressure/V.F. "(R)" (pressure divided by volume times frequency): changes in pressure may be caused by changes of air flow, i.e. either changes of volume or of breathing frequency or of both. Elimination of this disturbing influence was attempted by calculating the mean pressure necessary for 1 litre of air to pass through one chamber during respiration. A minimum and maximum plateau is also obtained for this parameter. The form of this curve is nearly the same as in the case of P.

The general aspect of all these curves shows an "S" shape as seen in drug receptor interactions.

Maximal response

If, following the administration of the lower concentrations, no reaction can be measured, it may be due to the fact that the reaction cannot be detected by the technique, or that no reaction occurs at all. Attempts to differentiate between these two possibilities in the clinical experiments seem rather difficult. However, on the administration of higher concentrations, a limit of responsiveness is reached with a certain concentration: administration of higher concentrations does not result in an increased effect.

This limit of responsiveness is due to the physiological limitations of the system involved (e.g. the limit of the reaction is dependent on the maximal effort developed by the thoracic respiratory muscles) and/or because all receptors are acted upon.

It is possible, as is customary in pharmacology, to express the response as a percentage of the maximal response obtained in the system used, and to plot this response against the logarithmic concentration (RIGGS, 1963).

The dose-response curve (fig. 6) demonstrates a steep slope in the mid-portion, with horizontal parts on both sides. The problem arises, at which point in the curve the ratio of the dose to response, has to be taken. It is clear that this ratio cannot be expressed in the

flat parts of the curve. However, it (dose-response ratio=nasal reactivity) can be defined explicitly in the steep mid-portion. The reactivity of the nasal mucosa can be defined more unequivocally, the steeper the mid-portion.

To illustrate the steepness of the dose-response curves obtained in this investigation, the range of concentration increase (viz. concentration steps) necessary to obtain a maximal response, has been calculated for the pharynx-nostril pressure gradient and tidal volume in 73 determinations of the nasal passage. The concentrations in mg/ml have been numerically indicated as a power of 2 ($= 2^x$). Let the highest concentration of histamine, which does not result in any effect be 2^{X_1} mg/ml, and the lowest concentration of histamine which results in a maximum response be 2^{X_2} mg/ml. The difference of the exponents $x_2 - x_1$ (= number of concentration steps) is an index for the steepness of the slope with regard to the maximal response.

In table 10 the mean values are given for the pressure and volume (viz. the mean difference between maximum and minimum values) for the different ranges of concentration steps necessary to obtain a maximal response.

The question arises whether the number of concentration steps necessary to obtain a maximal response for the pressure, runs parallel with the concentration steps involved to get a maximal response for the volume.

From table 10, it can be seen that the mean values for the pressure and volume, are practically the same for the different ranges of concentration steps. However, there exists a variability in concentration steps, indicating a great variability of the slopes in the mid-portion. For obvious reasons a range of concentration steps is not suitable to establish the reactivity of the nasal mucosa.

50 Per cent response

The reactivity of the effector organ (in this case the nasal respiratory mucosa) can also be expressed by the concentration of histamine needed to produce a 50 per cent of the maximal response in the parameters measured, viz. the pressure, volume and "R". However, the steepness of the slope has not been taken into account with this

Table 10

The range of concentration steps calculated in 73 determinations of the nasal passage, illustrating the steepness of the mid-portion of the response.

Number of concentration steps necessary to obtain a maximal response (from min. to max.)		1	2	3	4	5	6	7	8-9
	Number	7	10	16	8	9	14	4	5
Pharynx-nostril pressure gradient in cmH_2O									
	Mean value	23.9	11.3	8.5	6.6	4.3	4.4	3.3	2.4
	Index of slope (P/conc. steps)	23.9	22.5	25.6	26.7	21.7	26.7	23.5	21.0
	Number	5	16	15	10	10	6	4	7
Tidal volume in ml	Mean value	370	363	292	388	324	340	421	402
	Index of slope (V/conc. steps)	370	181	97	97	64	56	60	47

50 per cent criterion. Therefore, another criterion has been postulated for the reactivity of the nasal mucosa, namely, "$2P_I$" response.

$2P_I$ Response

This is defined as the lowest concentration of histamine applied (expressed as x in the x of 2^X mg/ml) at which a change in the pharynx-nostril pressure gradient twice the initial pressure value can be measured. In this case, the steepness of the slope is taken into account. This parameter, can therefore, be considered a more discriminating criterion for the definition of the nasal reactivity.

Observation

The different possible criteria have been compared and have been checked by clinical observation; for this purpose a final criterion has been suggested, viz. "O" = the lowest concentration, expressed as x of 2^X mg/ml histamine, causing a swelling of the nasal mucosa.

The mean values, indicating the mean values of the reactivity of the nasal mucosa using these different criteria in 73 determinations of the nasal passage, are compared in table 11.

Table 11

Comparison of the different criteria for the assessment of the histamine reactivity of the nasal respiratory mucosa.

CRITERIA	Number of determinations	Mean value of the histamine concentration*	Standard deviation**
50 % change in pressure	73	+ 0.04	2.35
50 % change in volume	73	— 0.25	2.20
50 % change in P/V.F.	73	+ 0.45	2.34
2 P_I	73	— 0.56	2.18
O	73	— 0.53	2.26

*) Concentration expressed as x of 2^x mg/ml. (exponents of 2 mg/ml).
**) The standard deviations of the mean values for the nasal histamine reactivity are practically the same. A strong correlation has been found between the values of the mentioned criteria.

As can be seen from table 11, the mean values obtained for "$2P_I$" and "O", are practically the same.

Summarizing, it can be said that the reactivity of the nasal respiratory mucosa can be expressed as x in:

(1) the x of 2^x mg/ml histamine/causing a maximal reaction in the parameters P, V, P/V.F(t);
(2) the x of 2^x mg/ml histamine/causing a 50 % of the maximal reaction in the parameters P, V, P/V.F(t);
(3) the minimal x of 2^x mg/ml histamine/causing a reaction of at least twice the initial pressure value (= "$2P_I$");
(4) the minimal x of 2^x mg/ml histamine/causing a swelling of the nasal mucosa (= "O").

From this comparison of the criteria, "$2P_I$" has been chosen to assess the reactivity of the nasal mucosa since:
(a) the steepness of the slope is taken into account (= more sensitive parameter);
(b) the mean values obtained for $2P_I$ and O, are practically the same (see table 11);
(c) few determinations have to be done, which is important, especially for investigations on a large scale.

Although the criterion $2P_I$ is arbitrarily chosen, its application for the determination of the nasal reactivity has been accepted for the reasons mentioned above and it *is* applied in all the clinical and epidemiological investigations which are discussed in the following chapters.

§ 5. Possible sources of error in the assessment of the histamine reactivity of the nasal respiratory mucosa

It is conceivable that by the application of histamine to the nasal respiratory mucosa in the manner described in § 3, a combined effect may occur from the pharmacological action of histamine, the mechanical irritation, and the influence of the solvent. In order to evaluate these effects the following studies (a, b) were performed.

(a) *The influence of mechanical irritation in the assessment of the nasal histamine reactivity*

Saline solution was applied to the nasal mucosa six times consecutively, exactly in the same way as in the case of histamine application, in 10 patients with known increased reactivity of the nasal mucosa to histamine. The results are summarized in the next table.

Table 12

The pharynx-nostril pressure gradient in cmH_2O after six consecutive applications of saline solution.

		Number of determination					
Pat. No.	P_I	P_1	P_2	P_3	P_4	P_5	P_6
1	8.4	8.4	8.5	8.4	8.3	8.4	8.5
2	5.6	5.6	5.6	5.6	5.5	5.6	5.7
3	4.2	4.2	4.2	4.2	4.2	4.2	4.2
4	3.5	3.5	3.5	3.5	3.5	3.6	3.7
5	2.8	2.8	2.9	2.9	3.0	2.9	2.9
6	4.3	4.3	4.4	4.5	4.4	4.4	4.5
7	5.6	5.6	5.5	5.6	5.6	5.6	5.6
8	3.9	3.9	3.8	3.9	4.0	4.0	3.9
9	3.7	3.6	3.7	3.7	3.7	3.7	3.7
10	2.8	2.8	2.9	2.9	2.9	2.9	2.9
Mean	4.5	4.5	4.5	4.5	4.5	4.5	4.6

P(1-6): The pharynx-nostril pressure gradient after the applications of saline solution.

P_I : Initial pharynx-nostril pressure gradient.

No changes were found in the pressure values obtained after repeated application of saline solution.

From the results the conclusion can be drawn, that the mechanical effect of repeated intranasal application can be neglected in assessing the reactivity of the nasal respiratory mucosa to histamine by topical application.

(b) *The influence of the solvent in the assessment of the nasal histamine reactivity*

As stated before, histamine diphosphate is dissolved in distilled water, and to obtain a base-line value in determining the reactivity of the nasal respiratory mucosa to histamine, distilled water is used. In order to evaluate the effect of the solvent, distilled water was applied six times consecutively to the nasal mucosa, in 10 patients with known increased reactivity of the nasal mucosa to histamine. The results are given in table 13.

No change was found in the pressure values obtained after repeated application of distilled water.

Various investigators (PROETZ, LIERLE and MOORE, RICHTNER) stated that tap or distilled water are not suitable for the epithelium. RICHTNER said that isotonic salt solutions are best when considering solvents for antiseptics, adstringents and vasoconstrictors administered to the nasal mucous membrane.

Table 13

The pharynx-nostril pressure gradient in cmH_2O after six consecutive applications of distilled water.

		Number of detemination					
Pat. No.	P_I	P_1	P_2	P_3	P_4	P_5	P_6
1	2.8	2.9	2.8	2.8	2.9	2.8	2.8
2	3.5	3.5	3.5	3.6	3.5	3.6	3.6
3	3.2	3.2	3.3	3.2	3.2	3.2	3.3
4	5.5	5.5	5.6	5.6	5.7	5.7	5.7
5	6.5	6.5	6.5	6.6	6.5	6.5	6.5
6	7.2	7.2	7.2	7.2	7.2	7.2	7.2
7	5.6	5.6	5.7	5.6	5.5	5.5	5.5
8	4.2	4.2	4.2	4.2	4.2	4.2	4.3
9	4.6	4.7	4.7	4.6	4.6	4.7	4.7
10	5.2	5.3	5.3	5.3	5.3	5.3	5.3
Mean	4.8	4.9	4.9	4.9	4.9	4.9	4.9

P(1-6): The pharynx-nostril pressure gradient after the applications of distilled water.

P_I : Initial pharynx-nostril pressure gradient.

However, the conclusion can be drawn that the effect of distilled water as used in this method, on the nasal respiratory mucosa, can be completely neglected in the evaluation of the reactivity of the nasal respiratory mucosa to histamine by topical application.

§ 6. Topical application versus nebulization in the assessment of the histamine reactivity of the nasal respiratory mucosa

Although no irritative component influencing the results could be detected in the determination of the reactivity of the nasal mucosa to histamine by topical application, it is conceivable that application of histamine by aerosol might be less irritating for the patient than the topical application of histamine as described in § 3. It should be considered, however, that when histamine is applied by aerosol, it is also inhaled into the bronchial tree. In this connection, the following problems arise:

(1) whether there is a difference in the reactivity of the nasal mucosa determined by means of topical application, and inhalation of histamine per aerosol;

(2) whether any bronchial reactions occur when the reactivity of the nasal mucosa is determined by means of inhalation of histamine per aerosol, or by topical application.

Table 14

Comparison of the histamine reactivity (expressed as x of 2^x mg/ml) by means of topical application and per aerosol.

Reactivity of the nasal respiratory mucosa assessed by		
Topical Nasal Application	Application per aerosol	Difference
—2	1	—3
—4	—2	—2
—1	0	—1
—1	—1	0
—3	—1	—2
—4	—2	—2
—2	1	—3
—2	1	—3
—2	1	—3
2	5	—3
1	3	—2
—1	2	—3
		Mean —2.2

ad (1) To study this problem 12 patients with a marked reactivity of the nasal respiratory mucosa to histamine were selected. In determining the histamine reactivity by means of inhalation through one nostril for 30 sec., the histamine solutions were nebulized* with an air flow of 8 lit/min.. This comparison between the topical application and nebulization of histamine, was made under the same standardised conditions and at the same time, on different days. The results are shown in table 14.

Conclusion

Reaction of the nasal mucosa to histamine after topical application was found at significantly lower histamine concentrations compared with the reaction after histamine nebulization; the mean difference is 2.2 concentration steps. This difference can be explained by the fact that the total amount of histamine on the nasal mucosa is probably much higher in the case of topical application, than by nebulization.

*) "Original Wiesbadener Doppelinhalator"; Wiesbadener Inhalatoren-Vertrieb Karl Blümel, Wartestrasse 13, Wiesbaden, West Germany.

ad (2) To study the bronchial reaction during the assessment of the nasal reactivity 15 patients with nasal complaints (obstruction, sneezing and hypersecretion) and chest complaints (cough, sputum and dyspnoea) were selected and also 17 subjects without nasal and chest complaints. Some simple lung function data of the 15 patients are shown in table 15, whereas those of the 17 controls are represented in table 16. In the first session, the histamine reactivity of the bronchial tree was determined by oral inhalation of aerosols with successively increasing concentrations of histamine during 30 sec. (De Vries, 1963). The solutions of histamine were nebiluzed with an air flow of 8 lit/min.. The lowest concentration which causes a change of the Vital Capacity and/or FEV_1 of 10 % or more, was used as criterion for the bronchial reactivity. In the second session the histamine reactivity of the nasal respiratory mucosa and bronchial tree (as described above) was determined, simultaneously by means of an aerosol through one nostril.
Figure 7 shows a comparison of the bronchial histamine reactivity determined by means of oral and nasal inhalation.

This experiment shows that:

(a) bronchial reactions occur regularly in patients with chest complaints when the reactivity of the nasal respiratory mucosa is assessed by means of an aerosol instead of topical application;
(b) the reaction of the bronchi occurs at slightly higher concentrations in the case of nasal inhalation as compared with oral aerosol application of histamine.

In order to detect any reactions of the bronchial tree during the topical application of histamine, the following investigation was done. The pharynx-nostril pressure gradient and the oesophageal pharynx pressure gradient* were measured simultaneously during the assessment of the histamine reactivity of the nasal respiratory mucosa by topical application, in 25 patients with nasal and chest complaints. The bronchial histamine reactivity was established be-

* Two catheters (passed through one nasal chamber) were tied together so that when the oesophageal balloon was in place, the nasopharyngeal balloon laid at the level as described in § 3.

Table 15

Some simple lung function data of the 15 patients in whom the bronchial reactions were assessed after oral and nasal inhalation of histamine.

No.	Sex	Age	Bronchial histamine reactivity expressed as x of 2^xmg/ml	Predicted value* VC	Predicted value* FEV_1	Measured value VC	Measured value FEV_1	VC in % of predicted value	FEV_1 in % of predicted value
1	F	42	4	3630	2505	3200	2675	88	107
2	F	17	1	4270	3288	4100	3400	96	103
3	F	44	2	3220	2221	2525	1850	78	83
4	F	37	2	4060	2801	3550	2550	87	91
5	F	31	2	3720	2716	2600	2075	70	73
6	M	39	3	4180	2884	3575	2550	86	88
7	M	42	3	4610	3181	4150	3200	90	101
8	F	54	—1	2740	1781	2100	1400	77	79
9	F	33	3	3830	2681	3650	2625	95	98
10	M	17	3	4880	3806	4050	2875	83	76
11	M	18	3	4720	3634	4325	2700	92	74
12	M	25	—2	5320	4096	3350	1350	63	33
13	M	32	—1	4700	3431	5250	3450	112	101
14	F	47	—2	4310	2974	3025	1550	70	52
15	M	49	0	3825	2486	2725	1450	71	58

VC : Vital Capacity.
FEV_1: Forced Expiratory Volume in one second.
*) The values of the E.C.C.S., were not applied since they only refer to males.

Table 16

Some simple lung function data of the 17 controls (with normal reactions of the bronchial tree to histamine) in which the bronchial reactions were assessed after oral and nasal inhalation of histamine.

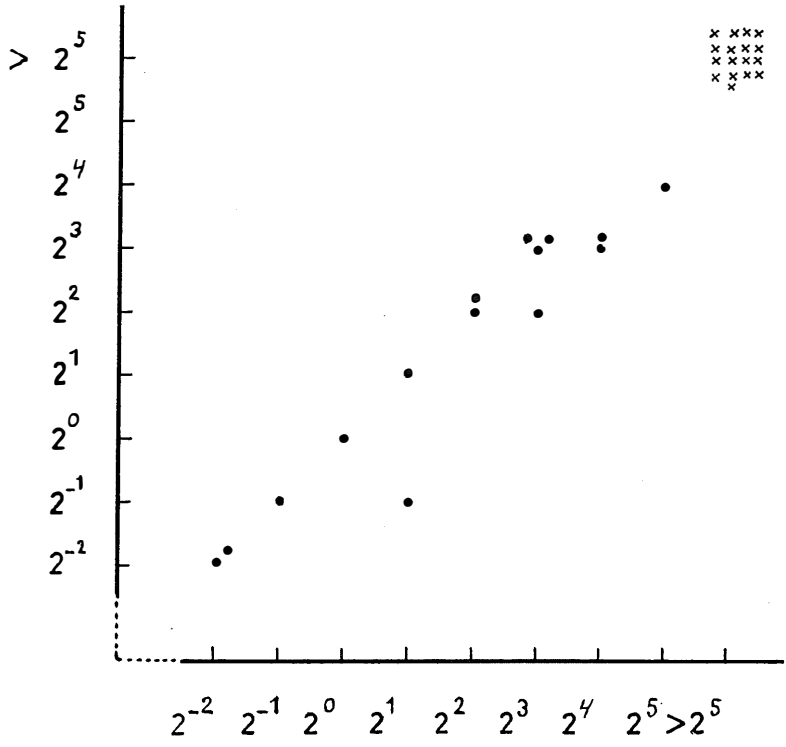
No.	Sex	Age	Predicted value VC	Predicted value FEV_1	Measured value VC	Measured value FEV_1	VC in % of predicted value	FEV_1 in % of predicted value
1	F	64	2600	1612	2375	1750	91	109
2	F	47	3070	1996	2800	2187	91	110
3	F	19	3740	2879	3200	2650	86	92
4	F	22	3740	2805	3550	3150	95	112
5	M	19	4130	3180	4856	3900	118	123
6	M	27	4400	3212	4650	3300	106	103
7	M	17	5200	4160	4050	2875	78	69
8	F	17	3300	2574	3700	3375	112	131
9	M	32	5630	4109	5175	4150	92	101
10	M	21	4560	3648	5650	4425	124	121
11	M	23	4880	3560	5350	4600	110	129
12	M	17	4450	3426	4150	3600	93	105
13	M	28	4110	3100	4250	3450	103	111
14	M	20	4720	3634	5700	4700	120	129
15	F	20	3630	2795	3900	3475	107	124
16	F	45	3700	2505	3475	2600	94	104
17	F	30	3390	2475	3650	3225	108	130

VC : Vital Capacity.
FEV_1: Forced Expiratory Volume in one second.

Fig. 7

Bronchial reactions during the assessment of the nasal histamine reactivity by inhalation per aerosol, in patients with nasal and chest complaints and in subjects without nasal and chest complaints.

Dose of histamine (as x of 2^x mg/ml) at which bronchial reactions occurred after oral inhalation.



Dose of histamine (as x of 2^x mg/ml) at which bronchial reactions occurred after nasal inhalation.

x = controls
• = patients

forehand by means of oral inhalation of histamine as described above. Some simple lung function data of these patients are shown in table 17.

The results obtained are gathered in table 18.

In this experiment no change of the oesophageal pharynx pressure gradient was noted in 22 out of 25 patients during the assessment of

Table 17

Some simple lung function data of the 25 patients with nasal and chest complaints in whom the pharynx-nostril- and oesophageal pharynx pressure gradients were simultaneously measured during topical histamine application to the nasal mucosa.

No.	Sex	Age	Bronchial reactivity expressed as x of 2^xmg/ml	Predicted value VC	Predicted value FEV_1	Measured value VC	Measured value FEV_1	VC in % of predicted value	FEV_1 in % of predicted value
1	F	29	0	3440	2580	2750	2100	80	81
2	F	57	1	3280	2394	2350	1400	72	58
3	M	45	2	4150	2864	4350	2700	105	94
4	F	25	3	4180	3219	4450	3700	106	115
5	F	20	1	3885	2991	3425	2000	88	67
6	M	33	1	4180	3051	3500	1475	84	48
7	M	34	—1	4475	3267	5300	3400	118	104
8	M	35	4	4925	3595	4950	3600	101	100
9	M	16	5	2390	1912	2100	1250	88	65
10	M	53	2	4610	2997	4300	1775	93	59
11	M	50	5	6600	4554	4950	1500	75	33
12	M	31	4	4400	3212	4150	2550	94	79
13	F	21	4	4450	3360	4250	3350	96	100
14	F	18	0	4400	3250	3500	2650	80	82
15	M	31	—1	4800	3430	5150	3450	107	100
16	F	19	1	4250	3250	2775	2100	65	65
17	F	21	5	3520	2816	3100	2150	88	76
18	M	21	0	5470	4103	5450	3200	100	78
19	M	43	5	4700	3713	4770	3625	101	98
20	F	34	5	3480	2475	2975	1850	85	75
21	F	41	2	4110	2836	2962	2525	72	89
22	F	64	4	3230	2003	3162	2425	98	121
23	M	17	5	4700	3431	3650	2525	78	74
24	M	53	2	4600	2997	4200	2250	91	75
25	F	20	0	4350	3250	3500	2840	80	90

VC : Vital capacity.
FEV_1: Forced Expiratory Volume in one second.

the nasal histamine reactivity by topical application. However, in 3 patients (No. 23, 24 & 25) a striking increase of the oesophageal pharynx pressure gradient was registered. The nasal respiratory mucosa was very sensitive and the bronchial tree of all 25 patients was found to be moderately sensitive to histamine.

When looking at the results, it can be seen that patient No. 23 had a relatively low bronchial reactivity to histamine (32 mg/ml = 5). However, after the administration of 1/8 mg/ml (= -3) histamine, which resulted in severe nasal obstruction (pharynx-nostril pressure gradient = 33.6 cmH_2O), an increase of the oesophageal

Table 18

Results of the influence of the nasal topical application of histamine on the oesophageal pharynx pressure gradient.

No.	B_{HR}	P_I	Pharynx-nostril pressure gradient at "2 P_I"	N_{HR}	Pe_1	Pe_2
1	0	2.8	11.2	—3	7.0	8.4
2	1	5.6	15.4	—3	4.2	4.2
3	2	5.6	16.8	—2	2.1	4.2
4	3	4.2	37.8	1	5.6	5.6
5	1	7.0	14.0	—2	4.2	6.3
6	1	7.0	26.6	—1	6.3	7.0
7	—1	7.0	21.0	1	7.0	6.5
8	4	3.5	12.6	—3	5.6	6.0
9	5	2.8	9.8	—1	4.2	4.2
10	2	7.0	12.6	0	4.0	4.2
11	5	4.2	14.0	—2	5.6	7.8
12	4	5.6	15.0	1	4.6	5.6
13	4	3.5	36.5	—2	4.9	4.5
14	0	4.2	19.6	2	5.6	5.8
15	—1	5.6	16.8	—1	4.2	5.8
16	1	3.5	21.5	3	4.0	4.0
17	5	2.8	10.5	—3	3.5	3.5
18	0	3.5	18.6	0	4.2	4.2
19	5	7.0	35.5	4	3.5	4.5
20	5	5.6	39.5	—2	3.5	3.5
21	2	3.5	15.0	2	4.5	6.5
22	4	5.6	18.6	—2	3.5	4.8
23	5	7.0	33.6	—3	4.2	11.2
24	2	5.6	39.2	—3	4.9	21.0
25	0	3.5	28.0	—2	4.6	21.0

B_{HR}: Bronchial histamine reactivity expressed as x of 2^x mg/ml
N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml
Pe_1 : Initial oesophageal pharynx pressure gradient in cmH_2O
P_I : Initial pharynx-nostril pressure gradient in cmH_2O
Pe_2 : Oesophageal pharynx pressure gradient in cmH_2O measured at $2P_I$
$2P_I$: Expressed as x of 2^x mg/ml

pharynx pressure gradient was also measured. A similar phenomenon was noted in the other 2 patients (No. 24 & 25) with a higher bronchial reactivity to histamine.

The question arises, why an increase of the oesophageal pharynx pressure gradient occurred in these 3 patients. It can be argued that this increase was due to a reaction of the lower respiratory tract caused by local absorption of histamine which reached the lower respiratory tract by means of the circulation. However, the histamine concentrations applied to the nasal mucosa were much lower than the histamine concentrations which caused changes in the bronchial

tree, measured as a decrease of the Vital Capacity and FEV_1. Therefore, this explanation seems rather doubtful.

Another possibility is that the immediate increase of pharyngeal pressure and the subsequent increase of the pressure in the lower respiratory tract, directly caused a calibre change in the bronchi.

Finally, neurogenic factors from the nose or nasopharynx may have been the cause of the increase of the oesophageal pharynx pressure gradient (see also chapter I, § 7).

§ 7. The anatomical structure and the nasal histamine reactivity

VAN DISHOECK (1940) pointed out that the pharynx-nostril pressure gradient is dependent upon the structure of the nose. Because of the complex nasal anatomical structure, it is difficult to formulate a simple criterion by which this factor can be taken into account in the evaluation of the reaction of the nasal mucosa to histamine.

The problem was approached in two ways.

a. The relationship between the initial pressure values and the reactivity of the nasal mucosa to histamine was investigated. It was assumed that the pharynx-nostril pressure gradient is related to the anatomical structure. It is known that this gradient is increased when the nasal chamber is narrower, in this case (leptorrhine) it is conceivable that the nasal passage will more rapidly be blocked as the result of a reaction to histamine than in the case of a wide nasal chamber (platyrrhine).

   In 244 patients (aged 10-60 years) with nasal and chest complaints (see Appendix I & III) the histamine reactivity of the nasal respiratory mucosa was determined by means of the method discribed in § 3.
   In table 19 a comparison of the initial pharynx-nostril pressure gradient and the histamine reactivity of the nasal respiratory mucosa is shown.
   No relationship between the initial pharynx-nostril pressure gradient and the reaction of the nasal mucosa to histamine was found [$\chi^2_{16} = 14.16$ ($0.2 > p > 0.5$)].
   It was realised, however, that the selection of the patients with nasal complaints, could exert a disturbing influence on the detection of a relation between the initial pharynx-nostril pressure

Table 19

Comparison between the initial pharynx-nostril pressure gradient and the histamine reactivity of the nasal respiratory mucosa in 244 patients with nasal and chest complaints.

	Pharynx-nostril pressure gradient in cmH_2O										
N_{HR}	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	Total
≧5	0	0	0	1	3	0	3	0	0	0	7
4	1	0	0	1	1	0	2	1	0	0	6
3	1	3	0	5	1	0	1	0	0	0	11
2	0	2	0	2	0	2	0	0	0	0	6
1	0	1	1	3	3	0	2	1	0	0	11
0	0	2	1	7	6	1	4	1	0	1	23
—1	0	8	5	15	9	3	5	4	4	0	53
—2	0	10	1	13	9	2	11	3	1	0	50
—3	0	5	1	11	11	0	6	7	2	1	44
—4	0	1	1	4	3	0	2	2	0	0	13
Total	2	32	10	62	46	8	36	19	7	2	244

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml

A statistical analysis was performed by a 5x5 table by grouping the nasal histamine reactivity as: 5 4 3 & 2; 1 0; -1; -2; -3 & -4; and the pharynx-nostril pressure gradient as indicated by the dotted lines.

gradient and the reaction of the nasal mucosa to histamine. Therefore, this problem was also approached in another way (b.)

b. The part played by the anatomical structure on the reaction of the nasal respiratory mucosa to histamine was analysed in 13 subjects with a normal nasal anatomical structure and in 25 patients with nasal septum deviations. The reactivity of the nasal mucosa to histamine was determined in all these subjects (with- and without nasal septum deviations) first on the right side and on the following day on the left.
The results obtained, are shown in the next table.

It can be assumed that the nasal mucosa on the left and right side of the nose is the same - which in fact is supported by the results of the control study (± same △ P and histamine reactivity, see table 21). If the reactivity of the mucosa plays the only rôle, the same degree of the reactivity of the nasal mucosa should be found on both sides in subjects with septum deviations.

A significant increase of the initial pharynx-nostril pressure gradient was found at the side to which the septum was deviated (narrowed side) [see table 20] (mean △ P deviated side = 8.8 cmH_2O; △ P at non-deviated side = 5.3 cmH_2O).

As can be seen in table 20 the reactivity of the nasal mucosa in

Table 20

The comparison of the histamine reactivity of the nasal respiratory mucosa and the initial pharynx-nostril pressure gradient (P_I) in the deviated and non-deviated side of the nose in patients with septum deviations.

	Deviated Side		Non-deviated Side	
No. of patients	P_I in cmH_2O	Reactivity expressed as x of 2^x mg/ml	P_I in cmH_2O	Reactivity expressed as x of 2^x mg/ml
1	7	4	6.0	3
2	8.6	—2	7.0	—1
3	5.6	1	5.6	1
4	6.5	0	3.8	0
5	7.0	0	2.8	6
6	15.6	—5	3.0	5
7	16.8	—5	4.2	—2
8	11.2	—4	5.6	—3
9	10.6	—4	4.2	—2
10	9.8	—4	6.0	—1
11	7.0	0	5.6	1
12	5.6	4	4.5	2
13	8.4	—1	5.6	0
14	7.5	3	5.6	5
15	7.0	0	3.5	2
16	6.8	—2	5.6	—1
17	7.0	—1	7.3	1
18	10.6	—3	5.6	0
19	12.5	—2	6.8	2
20	7.0	0	7.5	—1
21	11.4	—4	6.5	—1
22	7.0	3	6.8	3
23	5.6	0	4.2	1
24	9.8	—2	4.5	—1
25	8.5	—1	5.6	0
Mean	8.8	—1.0	5.3	+0.8

Table 21

The comparison of the histamine reactivity of the nasal mucosa and the initial pharynx-nostril pressure gradient (P_I) in the left and right side of the nose, in subjects with apparently normal nasal chambers.

	Right side		Left side	
No. of subjects	P_I in cmH_2O	Reactivity expressed as x of 2^x mg/ml	P_I in cmH_2O	Reactivity expressed as x of 2^x mg/ml
1	5.7	1	5.6	0
2	5.6	1	5.0	0
3	4.5	0	5.6	0
4	6.5	1	6.0	1
5	3.5	0	4.0	1
6	4.2	0	4.5	0
7	6.0	—2	5.6	—1
8	2.8	—3	4.0	—4
9	3.5	—1	3.5	—1
10	3.0	—2	3.5	—2
11	4.5	—3	5.0	—3
12	5.6	4	6.0	3
13	2.8	0	3.5	1
Mean	4.5	—0.3	4.7	—0.4

the deviated side, tends to be increased, viz. significant lower threshold values (expressed as x in the x of 2^x mg/ml) were obtained ($p = 0.01$). From the results it can be estimated that a shift of $\triangle$ P of 3.5 cmH_2O (8.8-5.3 cmH_2O) runs parallel with a shift of 1.8 concentration steps in the degree of histamine reactivity of the nasal mucosa.

Furthermore, in these subjects with slight deflections on inspection (No. 1-4, 11, 12, 17, 20, 22 & 23), the reactivity and P_I was found to be nearly the same as measured at the non-deflected side, while in the other subjects with a greater degree of septum deviation, reactions to lower histamine concentrations were found.

From the last experiment the conclusion can be made that the nasal reactivity is to a certain extent influenced by the anatomical structure (which is in accordance with the statement of VAN DISHOECK).

This seems to be in contrast with the results encountered in the 244 patients in which no relationship was found between the initial pharynx-nostril pressure gradient and the histamine reactivity of the nasal respiratory mucosa. However, it should be realised that in the last investigation (viz. nasal septum deviation) the conclusions were made from paired observations of the nasal passage in the same patient, whereas in the group of 244 patients, no direct comparison could be made.

The results of these investigations suggest that the anatomical factor is of no major disturbing influence in the assessment of the nasal histamine reactivity. A fundamental factor seems to be involved, which will be discussed in the following chapters.

§ 8. a. **Reproducibility of the nasal reactivity to histamine**
b. **Nasal cyclic changes**

ad a. The question arises as to how far the degree of the histamine reactivity of the nasal mucosa is reproducible. To approach this problem it was decided to determine the reactivity of the nasal mucosa to histamine, on five consecutive days. Conditions were standardised, namely:

i. the same technique and method were used;

ii. the procedure was carried out at the same time of the day.

Under these standardised conditions the experiments were repeated for 5 days in 10 patients and the results are summarized in the next table.

Table 22

Reproducibility of the histamine reactivity of the nasal respiratory mucosa in 10 patients with nasal complaints. The histamine reactivity is expressed as x of 2^x mg/ml

No. of patients	Days 1	2	3	4	5
1	—1	—1	—1	0	0
2	3	2	2	2	3
3	2	3	3	3	3
4	—2	—2	—1	—2	—1
5	1	1	1	1	0
6	—1	—1	0	—1	0
7	2	2	2	2	2
8	3	3	2	3	3
9	0	0	1	0	—1
10	1	1	0	1	1

Insignificant differences were found between the mean threshold values measured on different days (variance analysis).

These results are in accordance with the experiments carried out by other investigators on the reproducibility of the threshold values of histamine reactivity in the lower respiratory tract (De Vries, 1961; Knol, 1965).

To what extent a circadian rhythm exists is somewhat difficult to detect, because the effect of the provocation continues for several hours.

ad b. Another disturbing factor is the so-called nasal cycle. According to several authors (e.g. Heetderks, Stoksted, Ocura and Stoksted, Keuning, Cottle) a regular cycle of mucosal changes occurs alternating from one side to the other in the normal nose in such a way that the cycle has no effect on the total nasal passage and that under normal conditions it does not give rise to any sensation (Kayser, 1895), and that a normal pressure-volume curve is obtained (Stoksted, 1953).

Heetderks (1927) reported on a study of the nasal cycle in 60 persons under varying external conditions of temperature and

moisture. He found in 80 per cent of all the subjects studied, a regular nasal cycle with alternating changes in the lumina of the nasal chambers. The remaining 20 per cent of the subjects showed irregular fluctuations of the conchae on one side without alternating changes in the contralateral chonchae. In adolescents the cycle was very pronounced, decreasing with increasing age.

To estimate the influence of the so-called "alternating nasal cycle" on the nasal passage and therefore, indirectly on the histamine reactivity, subjects were studied in two groups:

a. five individuals without any respiratory (nasal or chest) complaints;
b. five patients with nasal complaints (obstruction, sneezing and hypersecretion) and chest complaints (cough, sputum and dyspnoea).

All the examinations were performed in the same room under comparable conditions of temperature and humidity. The experiments lasted for nearly 5 hours. All individuals examined were required to have an acclimatization period of about 45 min.. At time intervals of 30 min. the pharynx-nostril pressure gradient was first measured on the left side and immediately thereafter on the right side. After the 5 hour period the histamine reactivity of the nasal respiratory mucosa was determined at the right side of the nose.

An example of a "cyclic curve" of a patient with nasal complaints and a subject without nasal complaints is shown in fig. 8.

The pharynx-nostril pressure gradient measurements in the 5 patients with nasal and chest complaints are shown in table 23 while those of the 5 individuals without nasal and chest complaints are represented in table 24.

Results

1. In the 5 normals an "alternating cycle" of reaction was obtained. These alternating changes occurred in such a way that the normal nasal respiration was maintained without any complaints from the control persons during the 5 hours. The nasal mucosa of the controls showed a non-hyperreactive response to histamine.

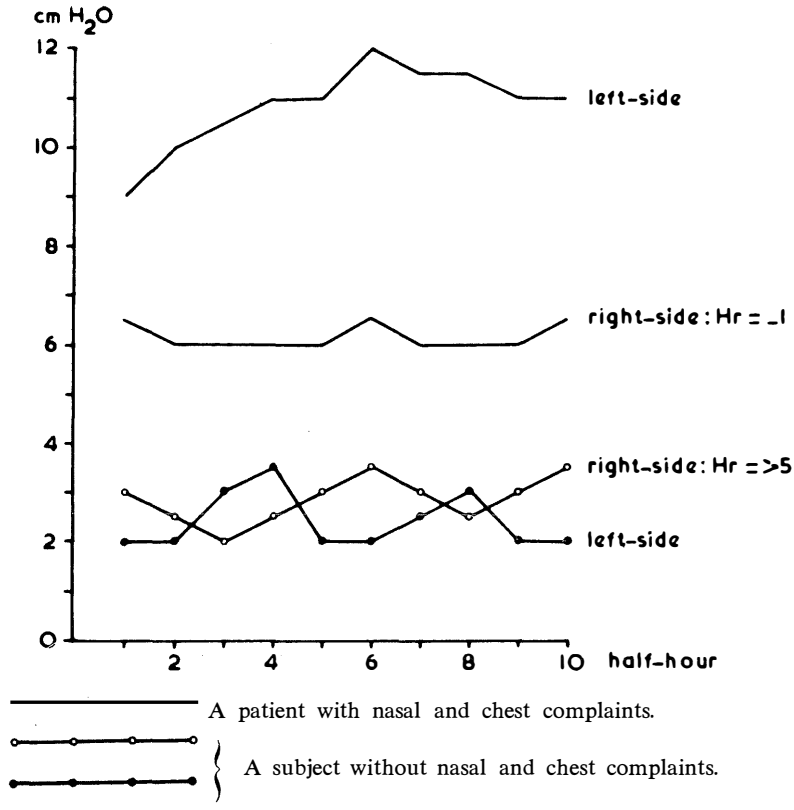


Fig. 8
"Nasal cycle".

Repeated determinations of the pharynx-nostril pressure gradient on the right and left side in a patient with nasal and chest complaints and in a subject without any complaints.

2. From the 5 patients with nasal and chest complaints no alternating cycle was obtained.

According to the literature, this "alternating nasal cycle" is controlled by the autonomic nervous system.

Table 23

Determination of the pharynx-nostril pressure gradient at time intervals of 30 min. in patients with nasal complaints (obstruction, sneezing and hypersecretion) and chest complaints (cough, sputum and dyspnoea).

	Pharynx-nostril pressure gradient in cmH_2O									
	Right side					Left side				
Time of	Patient No.					Patient No.				
determination	1	2	3	4	5	1	2	3	4	5
8.00 a.m.	6.0	11.0	6.5	9.0	6.5	7.5	6.0	5.5	7.0	9.0
8.30 a.m.	6.0	10.8	6.5	9.0	6.0	7.5	6.0	5.5	7.2	10.0
9.00 a.m.	6.0	11.0	6.5	9.0	6.0	7.5	5.8	7.0	7.2	10.4
9.30 a.m.	6.0	11.0	6.8	9.0	6.0	7.5	6.0	9.0	7.5	11.0
10.00 a.m.	5.8	11.0	7.0	8.7	6.0	7.5	6.0	10.0	7.5	11.0
10.30 a.m.	6.0	10.5	7.0	8.5	6.5	8.0	6.2	11.0	7.5	12.0
11.00 a.m.	6.3	10.6	7.3	8.2	6.0	9.0	6.0	12.0	7.5	11.5
11.30 a.m.	6.4	10.5	7.5	8.2	6.0	10.0	6.0	12.5	7.5	11.5
12.00 a.m.	6.5	10.7	7.5	8.0	6.0	10.5	5.6	12.5	7.3	11.0
12.30 a.m.	6.5	11.0	7.5	7.9	6.5	10.5	5.5	13.0	7.4	11.0
N_{HR}	−1	−2	0	−2	−1					

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

Table 24

Determination of the pharynx-nostril pressure gradient at time intervals of 30 min. in subjects without nasal and chest complaints.

	Pharynx-nostril pressure gradient in cmH_2O									
	Right side					Left side				
Time of	Subject No.					Subject No.				
determination	1	2	3	4	5	1	2	3	4	5
8.00 a.m.	3.0	3.0	5.0	4.0	3.0	4.0	6.0	4.5	1.8	2.0
8.30 a.m.	3.0	3.0	5.0	4.0	2.5	4.0	6.0	4.5	1.8	2.0
9.00 a.m.	3.0	3.5	4.8	4.5	2.0	4.0	5.0	4.5	1.5	3.0
9.30 a.m.	3.0	4.0	4.5	4.0	2.5	3.5	5.0	4.5	1.8	3.5
10.00 a.m.	3.5	4.0	4.3	3.5	3.0	3.0	5.0	4.3	2.0	2.0
10.30 a.m.	3.5	4.0	4.0	3.5	3.5	3.0	5.0	6.0	2.0	2.0
11.00 a.m.	4.0	4.0	4.0	3.5	3.0	3.0	4.5	7.0	2.3	2.5
11.30 a.m.	4.0	4.5	3.8	3.1	2.5	3.0	4.0	7.5	2.5	3.0
12.00 p.m.	4.0	5.0	3.0	3.0	3.0	3.5	4.0	7.5	2.8	2.0
12.30 p.m.	4.0	5.0	3.0	3.0	3.5	3.5	4.0	7.0	3.0	2.0
N_{HR}	>5	>5	>5	>5	>5					

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

Conclusion

1. In normal subjects without nasal complaints an alternating cycle of reaction occurs in the nasal mucosa of the two chambers.
2. In patients with nasal complaints this alternating nasal cycle is disturbed. In these patients an increased susceptibility to aspecific stimuli of the nasal respiratory mucosa exists.

3. The so-called nasal cycle seems not to be a disturbing factor in the reproducibility of the histamine reactivity of the nasal respiratory mucosa.

§ 9. Complications

In the course of the experiments carried out on the nasal respiratory mucosa in 244 patients, complications occurred in 2 subjects. It seems appropriate to describe the side reactions which occurred during the application of histamine to the nasal mucosa in these 2 patients.

In the first patient (female, 21 years, with nasal and chest complaints) no reaction was noted during the application of the lower histamine diphosphate concentrations, viz. 1/16, 1/8, 1/4 mg/ml. During the application of 1/2 mg/ml histamine diphosphate a reaction of the nasal mucosa was observed as a clear swelling and registered as an increased pressure and decreased volume. In order to obtain a dose-response curve, the next concentration, viz. 1 mg/ml was applied. Shortly after the application, the patient complained of headache and dizziness. A pulse rate of 120/min. was noted. Two minutes later she was in a state of excitement and shivering. The blood pressure was 110/60 mmHg, and the patient remained conscious. No bronchial obstruction was noted. CHVOSTEK's and TROUSSEAU's signs were negative. After 15 min. the "attack" subsided.

The other patient (male, 32 years, with nasal and chest complaints) showed a similar picture after the application of 2 mg/ml histamine diphosphate. However, in this patient the signs of CHVOSTEK and TROUSSEAU were positive and, later on he told that he had some tingling in his fingers, before the attack. This attack of hyperventilation with a "tetany-like" picture, lasted for 45 min., despite breathing in a bag.

In the following chapter the clinical significance of the nasal histamine reactivity will be discussed with regard to the clinical syndromes, epidemiological studies and the diagnostic point of view.

Chapter IV

CLINICAL SIGNIFICANCE OF THE NASAL HISTAMINE REACTIVITY

§ 1. Introduction

In the preceding chapter, the method and procedure for the assessment of changes in the nasal passage, the criteria for the nasal reactivity, the possible sources of error and the influence of the anatomical structure, have been discussed.

An important point concerning the clinical significance of the histamine reactivity, is the demonstration of a relationship between nasal complaints and the histamine reactivity of the nasal respiratory mucosa.

The results of a pilot study in a (clinical) group of patients will be discussed in the next paragraph.

§ 2. The relationship between the nasal complaints and the nasal histamine reactivity in patients with nasal and/or chest complaints

In the course of the experiments the nasal histamine reactivity was assessed in a large group of patients. This group consisted of 93 males and 145 females. The ages ranged from 10 to 70 years.

They were "selected" from out-patients attending the Division of Pulmonary Diseases of the Department of Medicine and the Department of Oto-Rhino-Laryngology of the State University Hospital, Groningen.

The complaints of the patients were of nasal and/or chest origin. Twelve patients had only chest complaints, the remainder, both. This heterogeneous group can therefore, not be considered as a random sample of a regional or otherwise definiable population. As the result of this restriction an epidemiological survey had to be done (see § 3).

Relation between the age and the degree of the nasal histamine reactivity

Figure 9 shows the distribution of the mean value of the reactivity of the nasal respiratory mucosa to histamine and the initial pharynx-nostril pressure gradient in different age groups. It appears that the difference in histamine reactivity is not correlated with the mean initial pharynx-nostril pressure gradient, which, as can be seen keeps at a constant level for the different age groups. This figure illustrates a difference in the nasal histamine reactivity: a high degree of histamine reactivity in the younger age groups and a lower degree in the older age groups. This difference is statistically significant ($p = 0.005$).

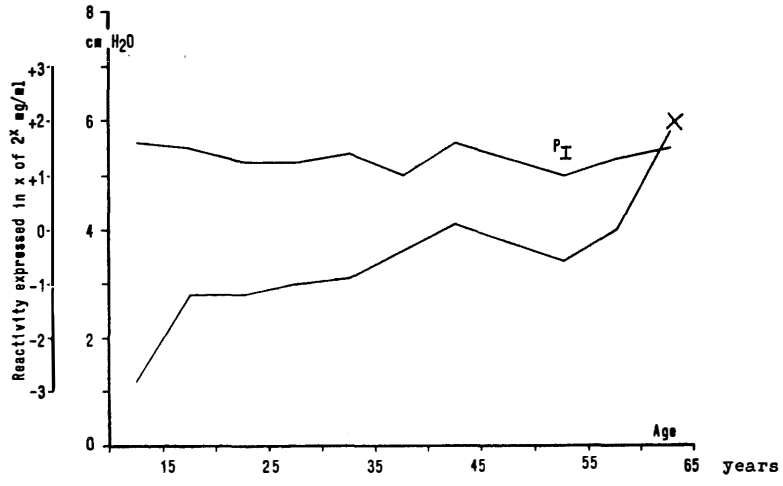


Fig. 9

Diagrammatic representation of the nasal histamine reactivity and the initial pharynx-nostril pressure gradient in the various age groups of 238 patients.

x : The minimal x of 2^x mg/ml histamine/causing a reaction of at least twice the initial pharynx-nostril pressure gradient("$2P_I$").

P_I: The initial pharynx-nostril pressure gradient in cmH_2O.

Relation between the nasal complaints and the nasal histamine reactivity

The relationship between the nasal complaints and nasal histamine

reactivity is shown in table 25. The data regarding the nasal complaints were obtained by means of the questionnaire given in Appendix I. The results are arranged according to age and histamine reactivity; the nasal complaints were scored as follows:

one for obstruction
one for sneezing
one for hypersecretion

Table 25

Relationship between the number of nasal scores and the histamine reactivity of the nasal respiratory mucosa, in 238 patients with nasal and chest complaints.

Age groups	No. of patients	N_{HR}	Nasal scores 0* & 1	2	3	Chi-square	Significance
10-19	59	$\geqq -1$ $\leqq -2$	11 4	11 8	4 21	$\chi^2_2 = 14.64$	$p = 0.001$
20-39	100	$\geqq -1$ $\leqq -2$	14 6	19 13	16 32	$\chi^2_2 = 9.58$	$p = 0.01$
$\geqq 40$	79	$\geqq -1$ 0 & 1 $\leqq -2$	15 5 3	9 11 7	3 9 17	$\chi^2_4 = 21.273$	$p = 0.001$

N_{HR} = Histamine reactivity of the nasal respiratory mucosa expressed as x of 2^x mg/ml.

* 12 patients without nasal complaints: 1 in the age group 10-14; 3 in the age group 25-29; 2 in the age group 35-39; 1 in the age group 40-44; 2 in the age group 50-54; 1 in the age group 55-59 and 2 in the age group $\geqq$ 60.

From the above table, a statistically significant relationship was found between the nasal scores and the reactivity of the nasal respiratory mucosa to histamine, in the different age groups. This relationship suggests a clinical significance of the histamine reactivity. However, this explanation cannot be generalized, since patients were merely selected for this investigation and control persons were not included. Therefore, an investigation of individuals, not selected according to nasal complaints seems necessary for a more generalized judgement on the clinical significance (see § 3).

§ 3. The relationship between the nasal complaints and the nasal histamine reactivity in two random samples of "normal" populations

1. *Introduction*

In the past, epidemiology was chiefly concerned with infective diseases; at present, however, epidemiological methods are also frequently applied to study the prevalence, natural history and aetiological factors of chronic non-infective diseases (e.g. cardio-vascular and chronic non-specific lung disease).

In the preceding paragraph, the reasons for an epidemiological investigation concerning nasal complaints and the nasal histamine reactivity have been mentioned. Before presenting the results of this epidemiological survey, a brief review of the literature concerning the epidemiology of nasal complaints, will be given. No epidemiological data have been found concerning the reactivity of the nasal mucosa.

2. *Prevalence of nasal complaints, a brief review of the literature*

FRANSSEN (1957) reported his findings regarding the prevalence of bronchial asthma, pollinosis, "vasomotor rhinitis", urticaria, eczema and infantile eczema in students and nurses. By means of an unspecified questionnaire he obtained his data from 338 males and 162 females (students and nurses). He reported (beside other results):

a. "vasomotor rhinitis" (with known cause) 4.7 % (total: 338 males and 3.0 % (total: 162 females);
b. "vasomotor rhinitis" (without known cause) 5.0 % (total: 338 males) and 1.2 % (total: 162 females);
c. pollinosis 5.0% (total: 338 males) and 1.3% (total: 162 females);
d. bronchial asthma 5.9 % (total: 338 males) and 2.5 % (total: 162 females).

In 1959 FLETCHER et al. studied the significance of respiratory symptoms in Post Office employees in London (see table 26).

FERRIS and ANDERSON (1962), studied the prevalence of chronic respiratory disease in a new Hampshire town. The dominant industry is pulp and paper mill for the manufacturing of craft paper products. "In addition, it operates a paper product tube mill and a chlorine plant". Other minor industries are: manufacturing of

canvas, rubber footwear and a knitting company. The survey was done in two parts: the first from January 1st till February 10th, 1961 and the second from June 1st till July 29th, 1961.
HOLLAND and REID (1965) reported their findings of an epidemiological study regarding the urban factor in chronic bronchitis. Two groups of men were selected for this survey, namely mail-van drivers and vehicle-maintenance men in central London, and secondly men employed, either as mail-van drivers or engineering workers.

Another epidemiological study has been reported by SHARP et al. (1965) concerning the prevalence of chronic bronchitis in an American male urban industrial population.

The results of these four surveys regarding the prevalence of nasal complaints, are summarized in table 26.

HUHTI (1965) reported results regarding the prevalence of respiratory symptoms, chronic bronchitis and pulmonary emphysema in

Table 26
The prevalence of nasal complaints according to various authors.

Age groups	35-40	40-44	45-49	50-54	55-59	60-64	Total
Author:							
Fletcher et al. (1959) "Stuffy nose or catarrh" during							
—winter : males		37 %		47 %			192 men
females		35 %		29 %			192 females
—summer: males		23 %		27 %			
females		18 %		19 %			
Ferris & Anderson (1962) "Nasal catarrh" during							
—winter : males	23 %		34.9 %		28.0 %		334 men
females	14.5 %		19.0 %		26.5 %		426 females
—summer: males	20.5 %		28.9 %		21.7 %		
females	13.7 %		19.0 %		20.6 %		
Holland & Reid (1965) "Nasal catarrh"							
London (males)		23.9 %		34.3 %			London: 250
Country town (males)		24.3 %		20.8 %			Country town: 426
Sharp et al. (1965) "Nasal catarrh"							
—summer (males)				17.3 %			1887
—winter (males)				33.6 %			

Table 27
Prevalence of nasal catarrh in various age and smoking groups: males and females. (According to HUHTI, 1965).

Nasal catarrh 3 months in the year (22) males	Non-smokers		Ex-smokers		Cigarette smokers (cig./day) 1-14		15-24		25-		Total	
Age	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
40-44	2	6.5	3	7.7	1	4.0	10	20.0	1	5.0	17	10.1
45-49	3	10.3	2	9.5	4	20.0	8	18.6	7	26.9	24	17.1
50-54	1	4.3	1	2.6	1	3.8	9	18.0	—	—	12	7.6
55-59	2	7.4	2	8.7	1	3.7	4	12.9	1	10.0	10	8.5
60-64	—	—	1	5.3	2	20.0	4	23.5	2	18.2	9	12.9
40-64	8	6.6	9	6.4	9	8.3	35	18.3	11	12.9	72	11.0

Nasal catarrh 3 months in the year (22) females	Non-smokers		Ex-smokers		Cigarette smokers (cig./day) 1-14		15-		Total	
Age	No.	%	No.	%	No.	%	No.	%	No.	%
40-44	7	4.6	1	8.3	3	7.9	—	—	11	5.3
45-49	7	4.6	1	(14)	2	10.5	—	—	10	5.6
50-54	10	6.0	—	—	—	—	1	(50)	11	6.0
55-59	—	—	—	—	—	—	—	—	—	—
60-64	5	5.7	—	—	—	—	—	—	5	5.4
40-64	29	4.1	2	6.7	5	6.5	1	(14)	37	4.5

a rural population and gave the following numbers regarding nasal complaints (see table 27).

Less conclusive reports coming from other investigators, are the following.

WOOD and MEADOWS (1963) reported their results of a survey of respiratory symptoms among 177 workers in a factory and gave the following numbers regarding nasal complaints:

	Yes	No	Unknown
"Nasal catarrh" winter only	17	—	—
Throughout the year	42	73	33

EL MEHAIRY et al. (1965), carried out a survey regarding the incidence of allergic disorders in 1350 subjects and found "allergic rhinitis" in 14.6 %.

However, in all the epidemiological studies reported, no specified nasal standardised questionnaire has been found.

Summarizing the scanty epidemiological reports as represented above concerning the prevalence of nasal complaints, the following remarks can be made.

1. No "clear" age effect seems to be involved in the prevalence of nasal complaints ("nasal catarrh") in subjects over 40 years of age.
2. A predominance of males exists.
3. An environmental factor seems to be involved ("nasal catarrh": London vs. country town).
4. A seasonal influence appears to be of importance (higher percentage of "nasal catarrh" during winter than in summer).
5. The effect of cigarette smoking seems not to be of importance (HUHTI).

Own survey

It was possible to carry out an epidemiological study concerning the problems discussed in the preceding paragraph, as a result of a field-survey regarding the prevalence of CNSLD*. The investi-

* The survey regarding the prevalence of CNSLD has been carried out by the Divisions of Pulmonary Diseases of the State University Hospitals of Groningen and Utrecht under the auspices of the National Health Research Council TNO.

gation concerning the nasal complaints and nasal histamine reactivity had to be adapted to the design of the CNSLD investigation. Only subjects over 40 years of age could be studied. This implies that the influence of the allergic factor (which plays a rôle especially in juvenile individuals), the nasal histamine reactivity and nasal complaints could not be investigated in all age groups, as could be done in the investigation of the patient group. On the other hand, however, it was possible to study the influence of the environment on the nasal complaints and the histamine reactivity of the nasal respiratory mucosa.

Method and materials

The survey was done in two communities: *Sellingen* (October 1st-8th, 1965) localized in an agricultural area free from industrial air pollution; and *Vlaardingen* (November 1st-8th, 1965), situated in an industrial polluted area.

By means of a detailed standardised nasal questionnaire (see Appendix II) data concerning nasal complaints were obtained in Sellingen from 1060 subjects (539 males and 521 females) and in Vlaardingen from 1188 subjects (651 males and 537 females) [total: 2248].

The method and procedure applied to assess the reactivity of the nasal respiratory mucosa to histamine were the same as described in chapter III. The histamine reactivity of the nasal respiratory mucosa was assessed at random on a voluntary basis in individuals interrogated concerning nasal complaints in Sellingen (67 males and 44 females) and Vlaardingen (74 males and 47 females) [one refusal in Vlaardingen]. The histamine concentrations applied in this survey are: 0.125 (-3), 0.5 (-1), 2 (1), 8 (3) and 32 (5) mg/ml (for the reduction of the concentrations see chapter VIII, § 2).

Results

From the data obtained in the two random samples, the following problems were analysed.

a. The prevalence of nasal complaints (i) and their distribution according to age (ii), sex (iii) and environment (iv).
b. The co-existence of the nasal complaints (obstruction, sneezing and hypersecretion).

Table 28

The prevalence of nasal complaints (given in percentage) in the two random samples

Nasal complaints	40-44 years				45-49 years				50-54 years				55-59 years				≧ 60 years			
	Sell.		Vl.		Sell.		Vl.		Sell.		Vl.		Sell.		Vl.		Sell.		Vl.	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
N-	76.7	62.2	63.4	72.8	74.3	78.7	57.2	71.4	65.5	76.0	68.5	52.8	64.5	74.2	60.2	61.7	55.3	79.7	55.5	70.3
N_s	17.2	27.0	19.4	12.9	21.4	16.8	23.7	9.2	22.4	16.7	16.9	17.9	21.9	21.8	16.9	11.1	30.8	14.6	22.2	13.2
N_p	4.3	9.8	12.5	10.0	4.3	3.5	14.5	11.8	8.6	4.2	10.5	17.9	10.4	3.0	15.7	22.2	11.7	5.6	16.0	6.6
N_m	1.7	0.8	4.7	4.3	0	0.9	4.6	7.6	3.5	3.1	4.0	11.3	3.1	1.0	7.2	5.0	2.1	0	6.2	9.9
O-	72.4	81.1	50.9	67.1	70.9	81.3	51.1	68.9	80.2	76.0	66.1	67.9	78.1	80.2	54.2	71.6	75.5	80.9	71.6	68.1
O_s	15.5	11.5	24.6	14.3	17.1	15.9	22.1	14.3	15.5	11.5	15.3	17.0	9.4	16.8	18.1	9.9	13.8	14.6	9.9	17.6
O_p	4.3	6.6	13.4	10.7	3.4	2.7	15.3	9.2	4.3	6.2	6.4	10.4	7.3	1.0	12.0	6.2	3.2	3.4	6.2	9.9
O_m	7.8	0.8	11.2	7.9	8.5	0	11.4	7.6	0	6.2	12.1	4.7	5.2	2.0	15.7	12.3	7.4	1.1	12.3	4.4
S-	75.0	78.6	71.1	77.8	69.2	88.4	67.9	79.8	66.4	81.2	75.8	74.5	67.7	78.2	71.0	72.8	63.8	83.1	70.3	76.9
S_s	18.1	15.6	17.7	11.4	25.6	8.0	15.3	11.8	23.3	12.5	12.9	10.4	25.0	20.8	9.6	18.5	25.5	11.2	12.3	9.9
S_p	2.6	4.9	7.8	5.0	4.3	1.8	13.7	5.0	6.9	3.1	7.3	10.4	5.2	0	13.3	7.4	5.3	3.4	12.3	8.8
S_m	4.3	0.8	3.5	5.7	0.9	1.8	3.1	3.4	3.5	3.1	4.0	4.7	2.1	1.0	6.0	1.2	5.3	2.2	4.9	4.4
Total numbers	116	122	232	140	117	113	131	119	116	96	124	106	96	101	83	81	94	89	81	91

N: sneezing
O: nasal obstruction
S: nasal hypersecretion
Sell.: Sellingen
Vl.: Vlaardingen
M: males
F: females

N-: no sneezing complaints
Ns: seldom sneezing complaints
Np: periodical sneezing complaints
Nm: sneezing most days of the year
the same holds true for "O" and "S"

c. The relationship between the prevalence of nasal- and chest complaints.
d. The prevalence of the histamine reactivity of the nasal respiratory mucosa and its relation to the nasal complaints in the two random samples.
e. The influence of age and sex on the histamine reactivity of the nasal mucosa.
f. The "normal" histamine reactivity of the nasal mucosa.
g. The relationship between the initial pharynx-nostril pressure gradient and the nasal histamine reactivity.

ad a. (i) *The prevalence of nasal complaints* (obstruction, sneezing, and hypersecretion)

The data concerning the prevalence of the separate nasal complaints are arranged according to age, sex and environment in table 28.

Fig. 10 illustrates the results, which are given in table 28.

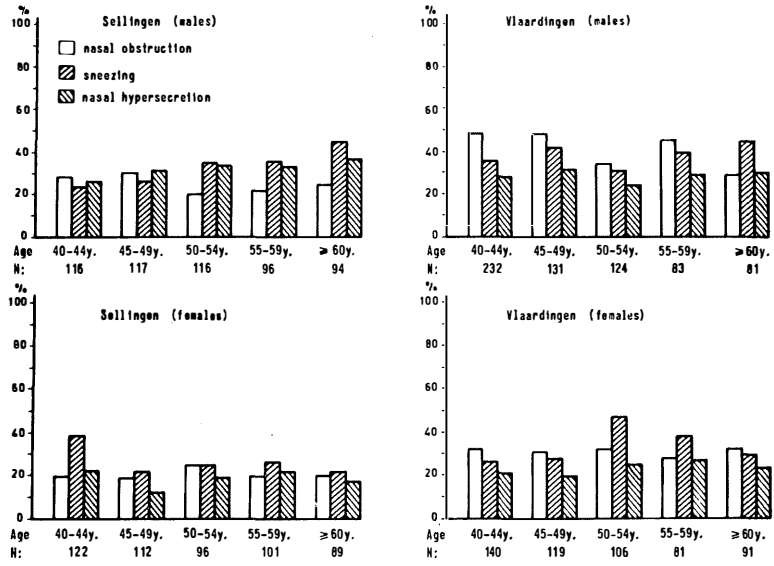


Fig. 10

Graphical representation of the data given in table 28.

As can be seen in the above figure, higher percentages for the nasal complaints were found in the random sample of Vlaardingen.

ad a. (ii) *The influence of the age factor on the nasal complaints*

The different age groups were divided into subgroups and these were then compared with respect to single nasal complaints. The subgroups are described in table 29 and were taken in order to rule out possible disturbing factors such as sex and environment. For the statistical analysis a χ^2 calculation on the basis of a 3×5 table was applied ("3" for the absence of complaints, seldom complaints, and periodical and most days; "5" for the 5 age groups). The results are shown in table 29.

From the results it can be concluded that there exists no relationship between the age and nasal complaints: obstruction, sneezing and hypersecretion. A more detailed description of the results are:

1. a tendency of nasal obstruction to decrease with increase of age in the males of Vlaardingen;
2. the gradual increase of sneezing complaints with increase of age occurring in the males of Sellingen; this pattern was not found in males of the industrial "polluted area";
3. the peak of sneezing (see fig. 10) in the females of Vlaardingen occurring in the 50-54 years age group. This increase of sneezing complaints was not encountered in the females of Sellingen. If this difference is due to the influence of air pollution then an increase of a peak of sneezing complaints has also to be found in the males of Vlaardingen which is not the case.

The influence of the menopause and "air pollution" may perhaps be considered. However, the peak of sneezing in the same female age group of Sellingen (rural area) was not encountered.

ad a. (iii) *The influence of sex on the nasal complaints*

For the statistical analysis a χ^2 calculation on the basis of a 2×3 table was used ("3" for no nasal complaints, seldom complaints, and periodical and most days; "2" for the males and females). The calculation was performed in comparable age groups and in the separate epidemiological groups — again in order to rule out possible disturbing factors such as environment and to a minor degree, the age factor. The results are shown in table 30.

Table 29

Results of the analysis of the influence of the age factor on the nasal complaints as represented in table 28.

Subgroup	χ^2	Degree of freedom	Significance in age group	Description of the difference
Nasal obstruction Sell. males	8.554	8	n.s.	—
Nasal obstruction Sell. females	13.370	8	n.s.	—
Nasal obstruction Vl. males	19.274	8	$0.02>p>0.01$	Tendency to decrease with age
Nasal obstruction Vl. females	3.610	8	n.s.	—
Sneezing Sell. males	17.841	8	$p = 0.025$	Gradually increase with age
Sneezing Sell. females	13.358	8	n.s.	—
Sneezing Vl. males	6.869	8	n.s.	—
Sneezing Vl. females	17.270	8	$p = 0.05$	Peak at 50-54 years
Nasal hypersecretion Sell. males	6.055	8	n.s.	—
Nasal hypersecretion Sell. females	14.673	8	n.s.	—
Nasal hypersecretion Vl. males	8.810	8	n.s.	—
Nasal hypersecretion Vl. females	7.273	8	n.s.	—

Sell.: Sellingen
Vl. : Vlaardingen

Table 30

Results of the analysis of the sex influence on the nasal complaints as represented in table 28.

Subgroup		χ^2	Degree of freedom	Significance	Remarks
Sell. nasal obstruction	40-44	2.676	2	n.s.	—
,, ,, ,,	45-49	7.711	2	$p = 0.025$	males>females
,, ,, ,,	50-54	5.121	2	n.s.	—
,, ,, ,,	55-59	8.041	2	$0.02>p>0.01$	males>females
,, ,, ,,	$\geqq 60$	2.441	2	n.s.	—
Vl. nasal obstruction	40-44	9.921	2	$p = 0.01$	males>females
,, ,, ,,	45-49	8.150	2	$0.02>p>0.01$	males>females
,, ,, ,,	50-54	0.484	2	n.s.	—
,, ,, ,,	55-59	5.514	2	n.s.	—
,, ,, ,,	$\geqq 60$	2.361	2	n.s.	—
Sellingen sneezing	40-44	5.794	2	$0.10>p>0.05$	females>males
,, ,,	45-49	0.761	2	n.s.	—
,, ,,	50-54	2.943	2	n.s.	—
,, ,,	55-59	5.567	2	n.s.	—
,, ,,	$\geqq 60$	14.424	2	$p = 0.001$	males>females
Vlaardingen sneezing	40-44	3.856	2	n.s.	—
,, ,,	45-49	9.461	2	$p = 0.01$	males>females
,, ,,	50-54	8.143	2	$p = 0.02$	females>males
,, ,,	55-59	4.257	2	n.s.	—
,, ,,	$\geqq 60$	1.298	2	n.s.	—
Sell. nasal hypersecretion	40-44	0.462	2	n.s.	—
,, ,, ,,	45-49	12.370	2	$0.002>p>0.001$	males>females
,, ,, ,,	50-54	6.072	2	$p = 0.05$	males>females
,, ,, ,,	55-59	5.952	2	$p = 0.05$	males>females
,, ,, ,,	$\geqq 60$	8.700	2	$0.02>p>0.01$	males>females
Vl. nasal hypersecretion	40-44	2.817	2	n.s.	—
,, ,, ,,	45-49	5.152	2	n.s.	—
,, ,, ,,	50-54	2.626	2	n.s.	—
,, ,, ,,	55-59	5.634	2	n.s.	—
,, ,, ,,	$\geqq 60$	1.004	2	n.s.	—

Sell.: Sellingen Vl. : Vlaardingen

Table 31

Results of the analysis of the influence of the environmental factor on the nasal complaints as represented in table 28

Subgroup		χ^2	Degree of freedom	Significance	Remarks
Male nasal obstruction	40-44	20.265	2	$p = 0.001$	Vl.>Sell.
,, ,, ,,	45-49	11.617	2	$0.005>p>0.001$	Vl.>Sell.
,, ,, ,,	50-54	11.950	2	$0.005>p>0.001$	Vl.>Sell.
,, ,, ,,	55-59	11.629	2	$0.005>p>0.001$	Vl.>Sell.
,, ,, ,,	$\geqq 60$	2.503	2	n.s.	—
Female nasal obstruction	40-44	8.226	2	$0.02>p>0.01$	Vl.>Sell.
,, ,, ,,	45-49	12.809	2	$0.005>p>0.001$	Vl.>Sell.
,, ,, ,,	50-54	1.789	2	n.s.	—
,, ,, ,,	55-59	13.025	2	$0.005>p>0.001$	Vl.>Sell.
,, ,, ,,	$\geqq 60$	5.538	2	n.s.	—
Male sneezing	40-44	8.787	2	$0.02>p>0.01$	Vl.>Sell.
,, ,,	45-49	14.241	2	$p = 0.01$	Vl.>Sell.
,, ,,	50-54	1.248	2	n.s.	—
,, ,,	55-59	2.85	2	n.s.	—
,, ,,	$\geqq 60$	3.013	2	n.s.	—
Female sneezing	40-44	8.594	2	$0.02>p>0.01$	Sell.>Vl.
,, ,,	45-49	13.583	2	$0.005>p>0.001$	Vl.>Sell.
,, ,,	50-54	17.069	2	$p = 0.001$	Vl.>Sell.
,, ,,	55-59	20.860	2	$p = 0.001$	Vl.>Sell.
,, ,,	$\geqq 60$	5.408	2	n.s.	—
Male nasal hypersecretion	40-44	0.868	2	n.s.	—
,, ,, ,,	45-49	10.732	2	$p = 0.005$	Vl.>Sell.
,, ,, ,,	50-54	4.369	2	n.s.	—
,, ,, ,,	55-59	10.795	2	$p = 0.005$	Sell.>Vl.
,, ,, ,,	$\geqq 60$	5.579	2	n.s.	—
Female nasal hypersecretion	40-44	2.714	2	n.s.	—
,, ,, ,,	45-49	3.597	2	n.s.	—
,, ,, ,,	50-54	4.196	2	n.s.	—
,, ,, ,,	55-59	6.120	2	$p = 0.05$	Vl.>Sell.
,, ,, ,,	$\geqq 60$	4.390	2	n.s.	—

Sell.: Sellingen Vl. : Vlaardingen

It can be seen in table 30 that the males generally have more nasal complaints than the females (except the males from Vlaardingen of the 50-54 age group).

The results suggest that the sex influence is of more importance than the age factor in the prevalence of the nasal complaints.

ad a. (iv) *The influence of the environment on the nasal complaints*

For the statistical analysis a χ^2 calculation was again used on the basis of a 2×3 table ("3" for the absence of complaints, seldom complaints and periodical and most days; "2" for the different areas: Sellingen and Vlaardingen). The calculation was performed in comparable age and sex groups in order to rule out factors which might have disturbed the picture (see table 31).

From the above table, it can be seen that, the random sample from Vlaardingen presents more nasal complaints than that from Sellingen — that means that the influence of the environment might contribute to the prevalence of nasal complaints. Which evironmental factor has to be regarded as important, represents another problem. However, the fact remains that Sellingen is located in an agricultural area free from industrial air pollution, while Vlaardingen represents a typical example of an industrial polluted area. The difference between the two random samples exists chiefly in respect to the complaints of nasal obstruction and sneezing. In respect to hypersecretion there seems to be a less pronounced difference.

In the older age groups no difference was found between any of the nasal complaints.

ad b. *The co-existence of the nasal complaints (obstruction, sneezing and hypersecretion)*

Until now, the different nasal symptoms were considered separately. However, it was felt necessary to see whether the nasal complaints co-exist or not. The results of this analysis are summarized in table 32 (the expected values are also added).

Table 32

The analysis of the results showing the co-existence of the nasal complaints, obstruction (O), sneezing (N) and hypersecretion (S) in the two random samples (see table 28).

Observed (Obs) and Expected (Exp) values			Sellingen Age groups										Vlaardingen Age groups									
			40-44		45-49		50-54		55-59		≧ 60		40-44		45-49		50-54		55-59		≧ 60	
			Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp	Obs	Exp
Males	S-	N-	65	48.3	54	42.7	57	40.3	42	32.8	34	25.0	76	53.2	40	26.1	50	42.6	30	19.3	22	22.7
		N+	8	14.7	13	14.7	11	21.3	16	18.0	14	20.7	27	30.7	17	19.5	16	19.6	10	12.7	22	18.1
	O-	N-	6	16.1	9	19.0	12	20.5	8	15.6	9	14.2	8	21.6	6	12.3	8	13.6	1	7.8	8	9.5
	S+	N+	5	4.9	7	6.5	13	10.8	9	8.6	14	11.5	7	12.5	4	9.2	8	6.2	4	5.2	6	7.6
	S-	N-	10	18.4	11	17.5	4	10.0	7	9.2	6	8.1	34	51.4	15	24.9	18	21.8	15	16.2	9	9.0
		N+	4	5.6	3	6.0	5	5.3	0	5.0	6	6.6	28	29.7	17	18.6	10	10.0	4	10.7	4	7.2
	O+	N-	8	6.1	13	7.7	3	5.1	5	4.4	3	4.6	29	20.9	14	11.7	9	7.0	4	6.6	6	3.8
	S+	N+	10	1.9	7	2.7	11	2.7	9	2.4	8	3.7	23	12.1	18	8.8	5	3.2	15	4.4	4	3.0
		Total	116		117		116		96		94		232		131		124		83		81	

		N⁻	63	48.5	72	64.1	59	45.1	57	47.0	56	47.8	73	53.3	64	46.8	39	25.8	39	26.5	45	33.5
	S⁻	N⁺	22	29.4	13	17.3	8	14.2	12	16.3	9	12.1	14	19.9	14	18.7	25	23.1	10	16.4	8	14.2
	O⁻	N⁻	8	13.1	3	8.3	3	10.4	6	13.1	3	9.7	5	15.2	3	11.8	3	12.2	4	9.9	4	10.1
	S⁺	N⁺	6	8.0	4	2.2	3	3.3	6	4.5	4	2.5	2	5.6	1	4.7	5	10.9	6	6.1	5	4.2
Females		N⁻	4	11.3	11	14.6	7	14.2	8	11.6	9	11.3	15	26.1	9	21.1	8	12.2	2	9.9	11	15.7
	S⁻	N⁺	7	6.8	4	3.9	4	4.5	2	4.0	0	2.9	7	9.7	8	8.4	7	10.9	8	6.1	6	6.6
	O⁺	N⁻	1	3.1	3	1.9	4	3.3	4	3.2	3	2.3	9	7.4	9	5.3	6	5.8	5	3.7	4	4.7
	S⁺	N⁺	11	1.8	3	0.5	8	1.0	6	1.1	5	0.6	15	2.8	11	2.1	13	5.1	7	2.3	8	2.0
		Total	122		113		96		101		89		140		119		106		81		91	

Samples	Age Groups	χ^2 Males	χ^2 Females	Degree of freedom	Significance Males	Significance Females
	40-44	54.58	61.85	3	$p = 0.001$	$p = 0.001$
	45-49	22.90	20.93	3	$p = 0.001$	$p = 0.001$
Sellingen	50-54	45.77	65.14	3	$p = 0.001$	$p = 0.001$
	55-59	30.28	31.75	3	$p = 0.001$	$p = 0.001$
	$\geqq 60$	13.74	43.57	3	$p = 0.005$	$p = 0.001$
	40-44	40.15	77.16	3	$p = 0.001$	$p = 0.001$
	45-49	28.10	64.24	3	$p = 0.001$	$p = 0.001$
Vlaardingen	50-54	7.02	32.13	3	$0.10 > p > 0.05$	$p = 0.001$
	55-59	43.57	27.77	3	$p = 0.001$	$p = 0.001$
	$\geqq 60$	4.46	30.03	3	$0.5 > p > 0.25$	$p = 0.001$

O⁺: Nasal obstruction positive
O⁻: Nasal obstruction negative - the same holds true for sneezing (N) and nasal hypersecretion (S)

From table 32 it can be seen that a statistically significant co-existence of the nasal symptoms was found in the various age groups of the two random samples - except in the 50-54 and $\geqq$ 60 male age groups of Vlaardingen.

ad c. *The relationship between the prevalence of nasal- and chest complaints*

In chapter II, a co-existence of rhinopathy and chronic non-specific lung disease has been reported. It seems therefore important to analyse this relationship between nasal- and chest complaints.

For the statistical analysis a χ^2 calculation was used on the basis of a 2 $\times$ 2 table ["2" for the nasal (presence or absence) and "2" (presence or absence) of the chest complaints]. The nasal complaints were taken as positive when one of the nasal symptoms was present, viz. obstruction or sneezing or hypersecretion. The same holds true for the chest complaints (cough and/or expectoration of sputum, or breathlessness).

The results of this analysis are represented in table 33 and fig. 11.

Conclusion

Generally, a positive relationship was found between the nasal and chest complaints in the two random samples from Sellingen and Vlaardingen.

except: 1) in the $\geqq$ 60 male age group of Sellingen;
2) in the 50-54 female age group of Sellingen;
3) in the 45-49 and $\geqq$ 60 male age groups of Vlaardingen;
4) in the 5 female age groups (40-44, 45-49, 50-54, 55-59, $\geqq$60) of Vlaardingen.

ad d. *The prevalence of the histamine reactivity of the nasal respiratory mucosa and its relation to the nasal complaints in the two random samples*

First of all, the prevalence of the histamine reactivity in both epidemiological groups will be discussed and will also be compared with the results obtained from the patients (see § 2).

The distribution of the degree of the histamine reactivity of the

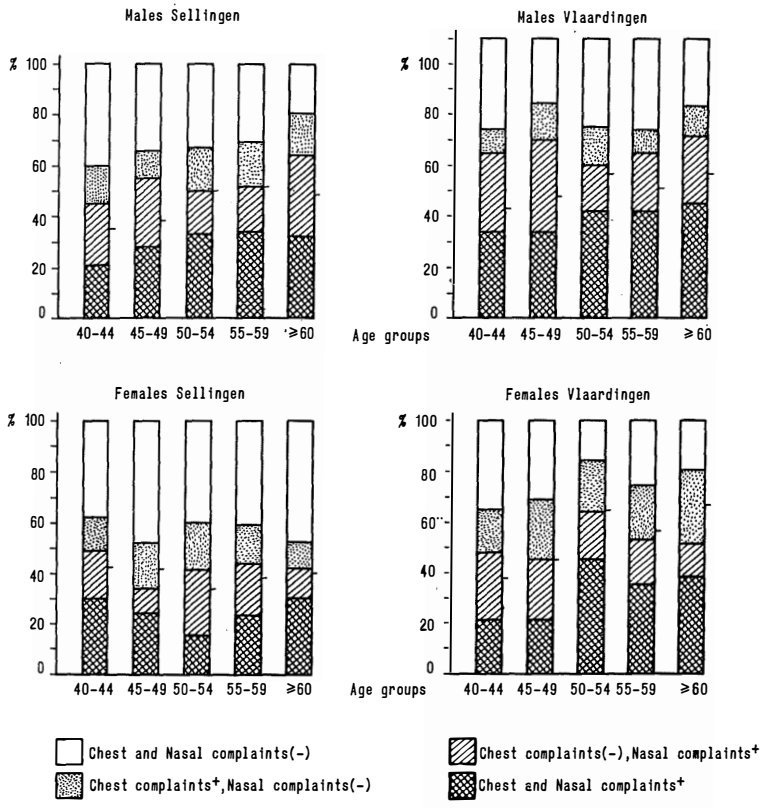


Fig. 11

A diagrammatic representation of the relationship between the prevalence of nasal- and chest complaints in the two random samples.

nasal mucosa in the random samples from Sellingen and Vlaardingen as well as that of the patient group is given in table 34.

A striking difference in the histamine reactivity was found between the two random samples and the patient group. The selection of the patient group explains this difference sufficiently. A marked difference (statistically significant) was also found between the two random samples ($\chi^2_3 = 17.833$, $p = 0.001$). This observation suggests an environmental influence (industrial air pollution) on the nasal histamine reactivity.

Table 33

Results of the analysis of the relationship between the prevalence of nasal- and chest complaints in the two random samples (see also fig. 11).

Subgroup		χ^2	Degree of freedom	Significance	Association between nasal and chest complaints
Males Sellingen	40-44	3.999	1	$p = 0.05$	positive association
	45-49	6.815	1	$p = 0.01$	„ „
	50-54	11.164	1	$p = 0.005$	„ „
	55-59	7.923	1	$p = 0.005$	„ „
	$\geqq 60$	0.223	1	n.s.	no association
Females Sellingen	40-44	15.403	1	$p = 0.001$	positive association
	45-49	18.585	1	$p = 0.001$	„ „
	50-54	1.251	1	n.s.	no association
	55-59	5.980	1	$p = 0.025$	positive association
	$\geqq 60$	21.335	1	$p = 0.001$	„ „
Males Vlaardingen	40-44	13.206	1	$p = 0.001$	„ „
	45-49	0	1	n.s.	no association
	50-54	10.750	1	$p = 0.001$	positive association
	55-59	8.967	1	$p = 0.005$	„ „
	$\geqq 60$	2.498	1	n.s.	no association
Females Vlaardingen	40-44	1.424	1	n.s.	„ „
	45-49	0.022	1	n.s.	„ „
	50-54	0.873	1	n.s.	„ „
	55-59	2.235	1	n.s.	„ „
	$\geqq 60$	2.373	1	n.s.	„ „

Table 34

Prevalence of the nasal histamine reactivity in the two random samples and in the patient group (§ 2)

Epidemiological groups	Histamine reactivity (expressed as x of 2^x mg/ml)												Total
	-3		-1		1		3		5		> 5		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Sellingen	0	0	7	6.4	21	19.1	14	12.7	4	3.6	64	58.2	110
Vlaardingen	7	5.6	16	13.9	36	28.8	19	15.2	7	5.6	40	32.0	125
Total	7	3.0	23	9.8	57	24.3	33	14.0	11	4.7	104	44.3	235
Patient group	57	25.4	103	46.0	34	15.2	17	7.6	6	2.7	7	3.1	224

Table 35

Comparison of the nasal scores in the two random samples and in the patient group

Epidemiological groups	Number of nasal scores								Total
	0		1		2		3		
	No.	%	No.	%	No.	%	No.	%	
Sellingen	61	55.5	24	21.8	15	13.6	10	9.1	110
Vlaardingen	52	33.6	43	34.4	30	24.0	10	8.0	125
Total	103	43.8	67	28.5	45	19.1	20	8.5	235
Patient group	12	4.9	41	16.8	73	29.9	98	40.2	244

Table 36

The relationship between nasal complaints and histamine reactivity of the nasal respiratory mucosa in the two random samples.

Epidemiological groups	Nasal histamine reactivity	Nasal complaints — No	%	+ No	%	χ^2_1	Significance	Association between N_{HR} and nasal complaints
Sellingen	≥ 5	54	49	14	13	40.549	p = 0.001	positive
	< 3	7	6	35	32			
Vlaardingen	≥ 5	22	18	25	20	5.114	p = 0.025	positive
	< 3	20	16	58	46			

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

Table 37

The nasal histamine reactivity of the subjects from the two random samples without nasal complaints and of the subjects in which the triads: nasal obstruction, sneezing and nasal hypersecretion occurs separately.

	The nasal histamine reactivity expressed as x of 2^x mg/ml												Total
	—3		—1		1		3		5		> 5		
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Sellingen													
Without nasal complaints	0	0	0	0	3	5	4	7	1	2	53	87	61
Nasal obstruction	0	0	1	17	1	17	0	0	0	0	4	67	6
Sneezing	0	0	2	18	5	46	1	9	0	0	3	27	11
Nasal hypersecretion	0	0	0	0	1	14	2	29	1	14	3	43	7
Vlaardingen													
Without nasal complaints	2	5	2	5	11	26	5	12	3	7	19	45	42
Nasal obstruction	2	10	2	10	7	33	4	19	1	5	5	24	21
Sneezing	1	6	4	25	3	19	3	19	0	0	5	31	16
Nasal hypersecretion	0	0	1	17	1	17	0	0	0	0	4	67	6

A similar pattern was found in respect to the distribution of the nasal complaints in the groups investigated. In table 35 the distribution of the number of nasal scores in the random samples from the Sellingen and Vlaardingen populations, as well as in the patient group, is recorded to illustrate this similarity.

This observation, immediately rises the question whether there is an association between the degree of the nasal histamine reactivity and the nasal complaints.

In the preceding paragraph it has been mentioned that the number of nasal scores in the patient group was found to be higher when the reactivity of the nasal mucosa to histamine was noted at the lower concentrations (higher degree of histamine reactivity). therefore, it seems necessary, to compare the number of nasal scores with the degree of histamine reactivity. In the next table (table 36) a comparison was made between the nasal complaints (1 score and higher for the presence of nasal complaints; score 0 for the absence of nasal complaints) and the degree of the nasal histamine reactivity in the epidemiological groups.

In the two random samples a statistically significant relationship was found between the nasal complaints and the histamine reactivity of the nasal mucosa.

However, when the two epidemiological groups are compared, the relationship between the nasal complaints and the nasal histamine reactivity, differs significantly ($\chi^2_3 = 27.745$; $p = 0.001$). The percentage of persons without nasal complaints and with a degree of nasal reactivity of $\geqq 2^5$ mg/ml histamine in the Sellingen group (49 %) was found to be nearly three times higher than that in the Vlaardingen group (18 %). On the other hand a predominance of persons with nasal complaints and a nasal reactivity of $\leqq 2^3$ mg/ml histamine was found in Vlaardingen (Sellingen 32 %, Vlaardingen 46 %). There are relatively more persons in the Vlaardingen group (16 %) without nasal complaints and a lower nasal histamine reactivity ($\leqq 2^3$ mg/ml) in comparison with the Sellingen group (6 %) (the nasal complaints of the negative group contribute 80 % of the nasal chisquare). This observation suggests that the nasal histamine reactivity is influenced to a higher degree

than the nasal complaints, by the environment (industrial air pollution.

The question arises whether a co-existence exists between the nasal histamine reactivity and a certain nasal complaint. This analysis, however, is opposed by the strong co-existence of the triads: obstruction, hypersecretion and sneezing (see ad b). The occurrence of an isolated (single) nasal complaint will therefore, not frequently be found.

The next table (table 37) shows the histamine reactivity of the nasal mucosa in a number of subjects without nasal complaints and a number of individuals with either nasal obstruction or hypersecretion or sneezing. The occurrence of a single nasal complaint in the patient group is rare and will not be analysed.

Table 37 illustrates the low frequency of occurrence of a single nasal complaint. The frequency of the isolated complaints within the two random samples, does not differ significantly. In view of the insignificant numbers, it is difficult to detect whether a difference in the degree of the nasal histamine reactivity exists in the different subgroups (with the isolated nasal complaints) of the two epidemiological groups. To approach this difficulty, the data of the two random samples were taken together and arranged according to < 32 mg/ml ($< 2^5$) and > 32 mg/ml ($> 2^5$) [see table 38].

Table 38

Relation between the nasal histamine reactivity (expressed as x of 2^x mg/ml) and the isolated nasal complaints (either obstruction or sneezing or hypersecretion) in the two random samples.

Occurrence of single complaints in Vlaardingen and Sellingen	Degree of nasal histamine reactivity	
	$\leqq 5$	$\geqq 5$
Nasal obstruction	18	9
Sneezing	19	8
Nasal hypersecretion	7	6

From the above table, no significant difference was found concerning the distribution of the nasal histamine reactivity in the subgroups with a single nasal complaint (3×2 table, $\chi^2_2 = 0.5$, n.s.). Therefore, the nasal histamine reactivity is not confined to a single nasal complaint. However, the nasal histamine reactivity was found

Table 39

Relationship between the nasal complaints and the nasal histamine reactivity in the two random samples arranged to age and sex.

Age group		40-44		45-49		50-54		55-59		60		40-49		50-65	
Nasal complaints		N-	N+	N-	N+	N-	N+	N-	N+	N-	N+	N-	N+	N-	N+
	N_{HR}														
Sellingen															
Males	≥ 5	7	1	7	1	4	0	5	1	5	4	14	2	14	5
	≤ 3	1	4	1	3	1	8	0	7	2	4	2	7	3	19
Females	≥ 5	0	1	7	0	6	2	8	2	5	2	7	1	19	6
	≤ 3	1	2	1	2	0	1	0	3	0	1	2	4	0	5
Vlaardingen															
Males	≥ 5	3	3	1	3	4	2	1	3	1	1	4	6	6	6
	≤ 3	6	15	0	8	4	9	1	7	1	4	6	23	6	20
Females	≥ 5	1	4	3	4	2	0	4	3	2	2	4	8	8	5
	≤ 3	3	5	2	4	1	2	1	1	1	3	5	9	3	6

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml
N- : Nasal complaints negative
N+ : Nasal complaints positive

to be increased in subjects with only nasal obstruction or only sneezing, in comparison with the subjects with nasal hypersecretion.

ad e. *The influence of age and sex on the histamine reactivity of the nasal mucosa*

The nasal histamine reactivity in the two random samples was arranged according to age and sex (see table 39).

Concerning the possible influence of age, on the nasal histamine reactivity, the different age groups were contracted into two groups for the statistical analysis (since the number of cases was limited), viz. 40-49, and $\geqq$ 50 years. The nasal histamine reactivity was also divided into two categories, viz. $\geqq 2^5$ mg/ml and $\leqq 2^3$ mg/ml. The analysis was done separately for the males and females of the two different epidemiological groups in order to eliminate possible disturbing factors. In such a way four 2x2 tables were obtained (see table 40).

Table 40

The distribution of the nasal histamine reactivity according to age and sex in the two random samples.

	Sellingen				Vlaardingen			
	Males		Females		Males		Females	
Age groups	40-49	$\geqq$ 50	40-49	$\geqq$ 50	40-49	$\geqq$ 50	40-49	$\geqq$ 50
N_{HR}								
$\geqq$ 5	16	19	8	25	10	12	12	13
$\leqq$ 3	9	22	6	5	29	26	14	9

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

From table 40 it appears that the distribution of the degree of the nasal histamine reactivity does not differ significantly in the different age groups. Therefore, the conclusion can be made that the influence of the age factor on the nasal histamine reactivity is insignificant in the two random samples investigated.

The sex influence on the nasal histamine reactivity was also analysed in the two epidemiological groups, but since no age influence was found, all age groups were taken together. The same categories of the histamine reactivity, viz. $\geqq 2^5$ mg/ml and $\leqq 2^3$ mg/ml were used for this analysis (2x2 tables).

Table 41

The distribution of the nasal histamine reactivity according to sex in the two random samples.

N_{HR}	Sellingen Males	Sellingen Females	Vlaardingen Males	Vlaardingen Females
≧ 5	35	33	22	25
≦ 3	31	11	55	23
Statistical Analysis		$\chi_1^2 = 4.49$ $p = 0.05$	$\chi_1^2 = 6.08$ $0.02 > p > 0.01$	

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

From table 41 a statistically significant difference was found between the males and females concerning the distribution of the degree of the nasal histamine reactivity. In both random samples the values were found to be lower in the males than in the females. This finding suggests that the nasal histamine reactivity is influenced by the sex factor. This sex difference is probably connected with a higher percentage of nasal complaints in the males in comparison with the females. Therefore, an analysis was made to see if this difference still exists when a subdivision is made according to the absence and presence of nasal complaints (see table 42).

Table 42

The distribution of the nasal histamine reactivity according to sex in the two random samples when the absence and presence of nasal complaints have been taken into account.

	Sellingen				Vlaardingen			
	N_c—		N_c+		N_c—		N_c+	
N_{HR}	Males	Females	Males	Females	Males	Females	Males	Females
≧ 5	28	26	7	7	10	12	12	13
≦ 3	5	2	26	9	12	8	43	15
Significance	n.s.		n.s.		n.s.		n.s.	

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.
N_c—: Nasal complaints absent.
N_c+: Nasal complaints present.
n.s. : Not significant.

When the nasal complaints are taken into account no sex difference can be demonstrated.

ad f. *The "normal" histamine reactivity of the nasal mucosa*

It seems reasonable to establish the "normal" value of the histamine reactivity of the nasal mucosa in "normal" subjects.

Different levels of normality are supposable (Donleben) and the final values will depend on the criteria applied. In this study no objective criteria (e.g. aspect of nasal mucosa) are available since only data from the history were recorded.

It is obvious that the definition of the "normals" on the basis of data from the history without objective observations, is not so precisely as it is in the case when both the history and objective observations are available. Nevertheless, an effort was made to obtain an estimation of the "normal" value of the nasal histamine reactivity in subjects without nasal complaints.

For values to be acceptable as "normal" the suggestion is made, that beside "normal" individuals also a "normal" environment has to be present, therefore, only the subjects from Sellingen without nasal complaints were chosen. The choice of "normal" values is of course arbitrary. In the folowing table the percentage of persons reacting at a concentration which may be considered as the "normal" value, is given; the subjects without nasal complaints in the random sample from Vlaardingen are also added.

Table 43

Prevalence of persons without nasal complaints with "normal" histamine reactivity of the nasal mucosa with different postulated normal values.

"Normal values" in mg/ml	% normal cases	
	Sellingen	Vlaardingen
> 32	87	45
$\geqslant 32$	88	52
$\geqslant 8$	95	64
$\geqslant 2$	100	90
$\geqslant 0.5$	100	95
$\geqslant 0.125$	100	100

In this case, the "normal" concentration value seems to be 8 mg/ml histamine, since 95 % of a normal population (Sellingen) reacts at 8 mg/ml or above.

ad g. *The relationship between the initial pharynx-nostril pressure gradient and the nasal histamine reactivity*

The relationship between the initial pharynx-nostril pressure gradient and the histamine reactivity has already been mentioned in chapter III.

In table 44 the initial pharynx-nostril pressure gradient of 235 subjects from the two random samples is arranged according to the degree of histamine reactivity of the nasal mucosa.

Table 44

The relationship between the initial pharynx-nostril pressure gradient and the degree of nasal histamine reactivity in the two random samples (total 235)

	Initial pharynx-nostril pressure gradient							
N_{HR}	0-0.9	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	Total
Sellingen								
> 5	4	30	21	5	2	2		64
5		2	1	1				4
3		4	6	1	3			14
1		12	6	2		1		21
— 1		6		1				7
Total	4	54	34	10	5	3		110
Vlaardingen								
> 5		13	21	4	2			40
5		2	4	1				7
3		4	9	6				19
1		8	15	7	3	3		36
— 1		1	7	6	1		1	16
— 3		1	3	2		1		7
Total		29	59	26	6	4	1	125

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

No statistically significant relationship was found between the initial pharynx-nostril pressure gradient and the nasal histamine reactivity (Sellingen $\chi^2_4 = 5.032$, $0.3 > p > 0.2$; Vlaardingen $\chi^2_4 = 8.355$, $0.10 > p > 0.05$). The results obtained from the epidemiological groups are in accordance with those obtained in the patient group.

Since a close association was found between the nasal histamine reactivity and the nasal complaints, the question arises whether an association exists too, between the nasal complaints and the initial pharynx-nostril pressure gradient.

In table 45 the initial pharynx-nostril pressure gradient of 235 subjects from the two random samples is arranged according to the presence and absence of nasal complaints.

Analysis from the data demonstrates no association between the initial pharynx-nostril pressure gradient and the nasal complaints in the two random samples. However, when the initial pharynx-

Table 45

The initial pharynx-nostril pressure gradient of 235 subjects assessed at random and arranged according to the presence and absence of nasal complaints.

	Initial pharynx-nostril pressure gradient							
	0-0.9	1-1.9	2-2.9	3-3.9	4-4.9	5-5.9	6-6.9	Total
Sellingen								
Without nasal complaints	3	28	16	7	4	3	0	61
With nasal complaints	1	26	18	3	1	0	0	49
Total	4	54	34	10	5	3	0	110
Vlaardingen								
Without nasal complaints	0	11	24	6	1	0	0	42
With nasal complaints	0	18	35	20	5	4	1	83
Total	0	29	59	26	6	4	1	125

nostril pressure gradient is compared in the two random samples, a significant difference exists ($\chi^2_3 = 23.2$; $p = 0.001$). In the random sample from Vlaardingen, a mean pharynx-nostril pressure gradient of 2.7 cmH_2O was found, while in the random sample of Sellingen, a mean pharynx-nostril pressure gradient of 2.2 cmH_2O was found.

A significant difference in the initial pharynx-nostril pressure gradient between the groups of Sellingen and Vlaardingen was also found in those, without nasal complaints ($\chi^2_2 = 10.313$; $p = 0.05$). The difference between the mean values, is insignificant (0.5 cmH_2O). The meaning of this difference in the initial pharynx-nostril pressure gradient between the two epidemiological groups, however, remains obscure.

Chapter V

EFFECT OF DIFFERENT PHARMACOLOGICAL AGENTS ON THE NASAL RESPIRATORY MUCOSA

§ 1. Introduction

For many years, studies have been performed to identify and to find the properties of substances - called, chemical mediators. Despite intensive research, however, our knowledge at present concerning the mediation of reactions by these chemical substances in man, remains largely descriptive. Moreover, this is a field which is developing and therefore, definite conclusions cannot yet be drawn.

At present, it is known that the effect of histamine mimics symptoms such as seen in allergic and non-allergic reactions.

Allergic reactions

Already in 1927, LEWIS had noticed a profound similarity between the action of an allergen applied intradermally to allergic individuals and that of histamine. Later in 1941, KATZ and COHEN demonstrated that histamine shifts from the cells to the plasma on incubation of blood from allergic individuals with antigenic material.

KATZ (1942) demonstrated that histamine is released in the skin of an allergic individual after application of an allergen. SCHILD et al. (1951) proved that histamine is released from the lung tissues and bronchioles of individuals sensitized to pollen and house dust on contact with these allergens; the tissues from normal persons did not react to the house dust or pollen extracts.

It has also been reported that histamine is released from the blood cells of an allergic individual, following addition of allergens to the blood [VAN ARSDEL et al. (1958), MIDDLETON and SHERMAN (1961)].

"The basic theory for an allergic response - that the antigen-antibody reaction releasing histamine in a specific shock organ is the sole factor - is now being challenged. The histamine theory

unquestionably explains most, but not all, of the phenomena encompassed by allergy, particularly that of the immediate type ... and we have recently been introduced to other mediators ..." (PRIGAL, 1960).

Non-specific reactions

DUKE already in 1924 and 1925 applied the term "physical allergy" to designate nasal, bronchial and cutaneous reactions caused by such agents as cold, heat, mechanical irritation, etc.. These reactions he said simulate the symptoms of such affections as "pollen disease". Such "allergic-like" manifestations after exposure to physical agents have been confirmed by various investigators who also noted an increase of histamine after such an exposure [e.g. HORTON, BROWN ROTH (1936), ROSE (1941), HENDERSON, CODE and ROTH (1958), DUNÉR, PERNOW and STERKY (1960)].

Other mediators beside histamine, which have been suggested to be involved in such reactions are, according to the literature, bradykinin, kallikrein, leukotaxine, kallidin, 5-hydroxytryptamine, acetylcholine, heparin, slow reacting substance (SRS), etc..

" .... Pieces of indirect evidence suggest that plasma kinins like bradykinin are formed in the interstitial space not only in glands during functional vasodilatation but also in damaged and inflamed tissues" (LEWIS 1963).

In this chapter attention will be focussed on acetylcholine, 5-hydroxytryptamine, bradykinin (kallikrein and padutin®), histamine liberators, adrenaline and isoproterenol. A brief review will be given of certain effects of these substances, viz. the effect on:

— smooth muscle
— blood vessels
— capillary permeability
— mucous glandular secretion
— eosinophils

Finally, a comparison will be made between the effects of these substances and that of histamine on the nasal respiratory mucosa.

§ 2. Acetylcholine

Acetylcholine was first synthetized by BAEYER in 1867. Later, at the turn of the century, HUNT (1899) noted that the blood pressure is lowered by adrenal extracts from which adrenaline has been removed — and suggested that a derivative of choline was the vasodepressor agent. Many reports followed regarding pharmacological properties of acetylcholine, e.g. HUNT and TAVEAU (1906), EWINS (1914), DALE (1914) who introduced the term "parasympathomimetic". DALE observed that the effects of acetylcholine mimicked the responses to stimulations of parasympathetic nerves, and today, acetylcholine is wellknown as the chemical mediator of cholinergic nerve activity. Acetylcholine plays a vital rôle in the neural transmission in the peripheral nervous system (and possibly a part in the central nervous system). Propagated nerve impulses cause liberation of this compound at nerve endings. At this point acetylcholine activates the postsynaptic membrane, probably by altering ionic movements and so causing its depolarization.

The peripheral pharmacological properties of acetylcholine may be divided according to the types of receptors with which it can combine, namely, its muscarinic action on gland cells, smooth muscle and the heart; and its nicotinic action on the autonomic ganglia, the motor end-plates of skeletal muscle and adrenal medulla. Therefore, acetylcholine mimics the action of preganglionic autonomic as well as the peripheral postganglionic parasympathic nerve fibres. The muscarine-like actions of acetylcholine can be antagonized by atropine or other anticholinergic drugs. The nicotine-like actions can be counteracted by curare-like and ganglionic blocking agents.

Cholinergic fibres include (according to GROLLMAN, 1960):

a. all parasympathetic postganglionic fibres;
b. postganglionic sympathetic fibres supplying the sweat glands and uterus;
c. sympathetic as well as parasympathetic preganglionic fibres;
d. the vasodilators;
e. neuromuscular effector fibres.

Effect on (bronchial) smooth muscle

DALE (1914) pointed out that acetylcholine causes increased

intestinal movements, lacrimation, salivation, constriction of the pupil and constriction of the bronchioles. KALLOS and PAGEL (1937) produced asthmatic attacks by inhalation of acetylcholine per aerosol. CARMICHAEL and FRASER (1933) studied the effects of acetylcholine in man, and stated ".... a few seconds after the injection the heart rate would slow abruptly and simultaneously the subject experienced a sense of obstructed breathing, causing him to cough, or a feeling of constriction in the chest".

STARR et al. (1933) injected subcutaneously 20 mg acetyl-beta-methylcholine (metacholine) and caused an "asthmatic-like" attack in a young subject with a history of asthma.

HURTADO and KALTREIDER (1934) found a decrease in the vital capacity associated with a sensation of substernal constriction in two "normal" subjects after the intramuscular injection of acetyl-beta-methylcholine in doses of 15-30 mg respectively. MOLL (1940) administered subcutaneously 5-20 mg of acetyl-beta-methylcholine and produced in 23 out of 28 asthmatic patients, asthmatic attacks. MOLL suggested a local sensitivity rather than a generalized state of increased sensitivity.

CURRY (1947) studied the action of acetyl-beta-methylcholine and histamine on the respiratory tract in normals, pollinosis and asthmatic patients. The reaction of the tracheobronchial tree was measured by recording changes in the vital capacity. The histamine and acetyl-beta-methylcholine were administered by means of intravenous injection and nebulization.

CURRY found a slight reduction in the vital capacity after the intramuscular injection of 6 mg metacholine (mecholyl®). In a group of 11 patients with pollinosis, all had a slight reduction in vital capacity following the administration of this drug. The same occurred in 27 asthmatics. On administration of histamine he found a reduction in the vital capacity in 23 subjects, and in 22 of these cases the reduction was 5 per cent or greater. CURRY concluded that "it appears that patients with hay fever and asthmatic subjects were more reactive to mecholyl chloride than to histamine and that both drugs acted by different mechanisms on the hyperresponsive respiratory tract of these individuals". HERXHEIMER (1949) also reported "asthmatic attacks as the result of acetylcholine inhalation per aerosol".

More recently TIFFENEAU (1955, 1957, 1958), TOMORI et al. (1964), DE VRIES (1962, 1964) confirmed by experiments that acetylcholine inhaled per aerosol evokes bronchial obstruction in "asthmatic" and "bronchitic" patients.

The reported results suggest a direct action of aecetylcholine upon the smooth musculature of the respiratory system. Furthermore, acetylcholine produces reflex cough (TIFFENEAU, TOMORI et al., l.c.).

Many experiments done in vitro and in vivo, on different animal species, confirmed the fact that acetylcholine causes contraction of smooth muscle [e.g. FOGGIE (1937), MOUNT (1956), DALY DE BURGH (1957), BORST et al. (1957), FRASER (1957), BHOOLA et al. (1962), INNES (1962)].

Effect on blood vessels

The effect of acetylcholine on the blood vessels, has also been studied, both in animals and in man. When this substance is given to an experimental animal or man by the intravenous route, it causes vasodilatation [e.g. GADDUM and HOLTZ (1933), MCDOWALL (1947), GRANT (1950), DUFF et al. (1953), GROLLMAN (1960), HOLTON and RAND (1962), WRICHT (1964), AVIADO (1965)].

However, vasoconstriction has also been observed, especially after large doses [e.g. FLEISH (1930), VON EULER (1932), GADDUM and HOLTZ (1933), ALCOCK et al. (1935), KOTTEGODA (1953), BORST et al. (1957)].

Regarding the effect of acetylcholine on the pulmonary circulation, AVIADO (1965) stated: "the most direct approach by lung perfusion has resulted in the demonstration of both vasoconstriction and vasodilatation". He suggested that, since acetylcholine is known to stimulate several synaptic junctions, it is conceivable that it stimulates some axon reflexes which are largely vasoconstrictor in nature, however, AVIADO concluded, that the action of acetylcholine in the lung are vasodilatation and bronchoconstriction, rather than vasoconstriction. "Acetylcholine has achieved clinical pre-eminence as a pulmonary vasodilator" (FISHMAN, 1963).

No report was found concerning the effect of acetylcholine on the nasal blood vessels.

Effect on capillary permeability

It still remains to be demonstrated whether the permeability response of the capillaries, is mediated by acetylcholine.

Effect on mucous glandular secretion

Acetylcholine causes an increased secretion from glands innervated by parasympathetic nerves [e.g. GOODMAN and GILMAN 1956), EVANS (1956), DALY DE BURGH (1957), WILSON and SCHILD (1959), GROLLMAN (1960), LEWIS (1960), TOMORI et al. (1964)].

Effect on eosinophilic leukocytes

No evidence exists that acetylcholine has any chemotactic effect on eosinophils.

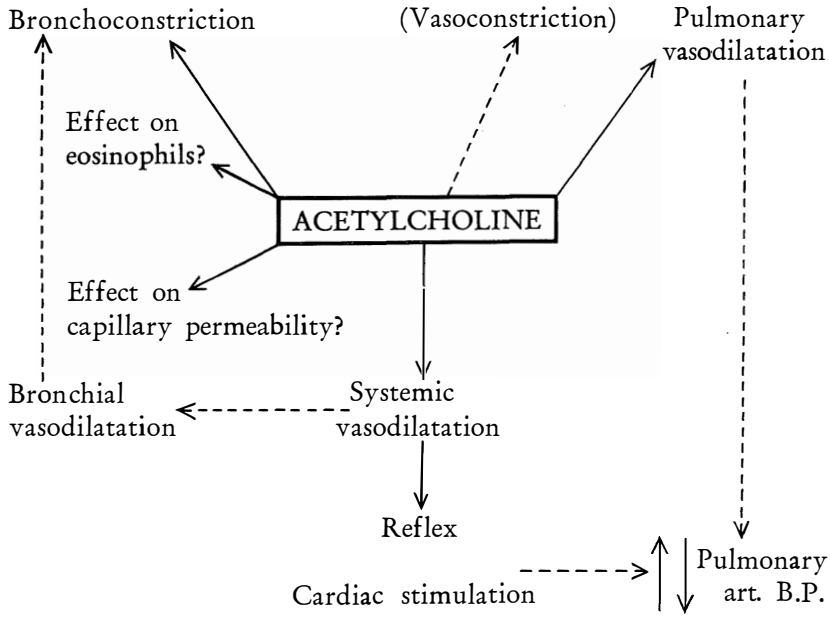


Fig. 12. Actions of Acetylcholine.
[After AVIADO, 1965 (slightly modified)].

Own clinical experiments

The effect of acetylcholine on the nasal respiratory mucosa was

Table 46

The comparison between the effect of histamine and acetylcholine on the nasal respiratory mucosa, in 20 patients with nasal and chest complaints.

No.	Sex	Age	Nasal Histamine Reactivity in mg/ml	Nasal Acetylcholine Reactivity in mg/ml	Observation: Histamine O	Histamine N	Histamine S	Acetylcholine O	Acetylcholine N	Acetylcholine S
1	M	35	0.25	> 512	+++	—	+	—	—	+
2	M	22	0.125	> 512	+++	+	+	—	—	+
3	M	34	0.25	512	+++	—	—	—	—	++
4	M	43	0.5	> 512	++	—	+	—	—	—
5	M	25	0.5	> 512	+++	+	+	—	—	+
6	M	27	0.5	> 512	+++	—	—	—	—	+
7	M	52	0.5	8	+++	—	+	++	—	++
8	M	54	0.25	> 512	+++	—	—	±	—	++
9	M	35	0.25	8	+++	—	+	+	—	+++
10	M	60	4	> 512	++	—	+	—	—	++
11	M	45	0.25	512	+++	—	+	+	—	++
12	M	19	0.5	> 512	+++	—	+	—	—	++
13	M	20	0.5	128	+++	—	+	+±	—	++
14	M	40	0.25	512	+++	+	++	±	—	++
15	F	18	32	> 512	+	—	+	—	—	—
16	M	58	0.25	> 512	+++	+	+	—	—	+
17	F	34	1	> 512	++	—	+	—	—	—
18	M	39	0.25	512	+++	+	++	±	—	++
19	F	28	0.125	> 512	+++	+	+	—	—	+
20	M	17	0.125	256	+++	+	+	+	—	++

O: Swelling of the mucosa
N: Sneezing
S: Hypersecretion

—: No reaction
+: Slight
++: Moderate
+++: Severe

Table 47

Some simple lung function data of the patients with nasal and chest complaints in whom the effect of histamine and acetylcholine on the nasal respiratory mucosa was compared.

		Predicted value		Measured value		VC and FEV_1 in % of the predicted value	
No.	B_{HR}	VC	FEV_1	VC	FEV_1	VC	FEV_1
1	4	4925	3995	4950	3600	101	90
2	0	4880	3700	3187	1775	65	48
3	4	4550	3325	4075	2125	90	64
4	1	3730	2425	2300	2300	62	95
5	1	4180	3219	3225	1625	77	51
6	6	4700	3572	4475	3825	95	107
7	—1	4350	3332	3670	1850	84	56
8	0	4250	2763	3350	2500	79	91
9	4	4925	3595	4950	3600	101	100
10	2	4165	2707	3800	2300	91	85
11	2	4150	2864	4350	2700	105	94
12	0	4720	3634	4325	2700	92	74
13	6	3900	3124	3425	2800	88	90
14	1	4250	2950	4250	2550	100	86
15	3	3885	2990	3750	2750	97	92
16	—1	4150	2573	3650	1900	88	74
17	6	3390	2475	2975	2850	88	115
18	3	5400	4200	5700	3850	106	92
19	2	4280	3200	3450	2000	81	63
20	0	4250	3300	3850	1850	91	56

B_{HR} : Bronchial histamine reactivity expressed as x of 2^x mg/ml.
VC : Vital Capacity.
FEV_1: Forced Expiratory Volume in one second.

studied in 20 patients with nasal complaints (obstruction, sneezing and hypersecretion) and chest complaints (cough, sputum and dyspnoea) with known histamine reactivity of the bronchial tree.

The histamine reactivity of the nasal respiratory mucosa was first determined, using the same method under the same standardised conditions, as described in chapter III. On the following day at the same time, and under the same standardised conditions the reactivity of the nasal respiratory mucosa to acetylcholine was determined.

The following concentrations of acetylcholine bromide (on a weight basis) were used: 1, 2*, 4, 8, 16, 32, 64, 128, 256 and 512 mg/ml (expressed as salt). The results obtained are given in table 46.

* 0.0088 Mol/lit.

Conclusion

When the effects of histamine and acetylcholine on the nasal respiratory mucosa are compared, the following conclusions can be drawn: *histamine caused a clear swelling of the mucosa* (sometimes accompanied by hypersecretion and sneezing), while *acetylcholine caused mainly hypersecretion* (sometimes accompanied by a slight swelling of the mucosa).

§ 3. **5-Hydroxytryptamine** (5-HT, Serotonin)

In extensive reviews PAGE (1954), ERSPAMER (1954, 1966), PAGE (1958), GARATTINI and VALZELLI (1965) have discussed in detail the properties and possible functions of 5-hydroxytryptamine in man and different laboratory animals. The most characteristic and important pharmacological property of 5-hydroxytryptamine seems to be its contractile effect on smooth muscle.

Effect on (bronchial) smooth muscle

COMROE et al. (1953) studied the reflex and direct cardiopulmonary effects of 5-hydroxytryptamine in 55 cats and concluded that 5-hydroxytryptamine given by rapid intravenous injection, produces profound reflex cardiopulmonary changes: bradycardia, hypotension, apnea, bronchoconstriction and pulmonary vasoconstriction.

A direct effect of 5-hydroxytryptamine on the bronchial muscle has been reported by various investigators [e.g. REID and RAND (1952), FREYBURGER et al. (1952), SINHA and WEST (1953), PAGE (1958), BROCKLEHURST (1960), GOTH (1961), GARATTINI and VALZELLI (1965)].

LEWIS (1960) and GROLLMAN (1960) said that 5-hydroxytryptamine is a powerful bronchoconstrictor and may be the cause of the asthmatic attacks in patients suffering from malignant carcinoids.

In 1953, HERXHEIMER studied the bronchial reaction to 5-hydroxytryptamine by means of inhalation in man. In 4 normal individuals inhalation of a 0.67 % aerosol of 5-hydroxytryptamine did not alter the vital capacity, nor the respiratory rate. However, in 3 of 6 asthmatic patients, inhalation of the same aerosol caused severe bronchial obstruction, abolished by isoprenaline. HERXHEIMER con-

cluded that 5-hydroxytryptamine possesses a bronchial action similar to that of histamine or acetylcholine. HAJÓS (1962) reported results concerning the rôle of 5-hydroxytryptamine in bronchial asthma. This investigator studied altogether 321 patients: (i) 246 asthmatics, (ii) 36 cases of "various allergic diseases other than asthma" and (iii) 39 non-allergic controls. 5-Hydroxytryptamine (2 % 5-HT sodium phosphate) was nebulized. A bronchospastic response to nebulized 5-hydroxytryptamine was found in 59 % of the asthmatics (i), 22 % of the "allergic group other than asthma" (ii), and 20 % of the "non-allergic" group (iii). The "non-allergic" group (iii) consisted of patients with "emphysema, chronic bronchitis, pneumonia, silicosis, cardiovascular disease, gastric ulcer, cirrhosis of the liver". HAJÓS concluded that 5-hydroxytryptamine is liberated during the antigen-antibody reaction and that the bronchospastic effect of 5-hydroxytryptamine is specific - "since it could be inhibited by serotonin antagonists (Deseril) in 75 % of the cases investigated. . .". On the other hand however, BROCKLEHURST (1962) unequivocally stated: "There is no evidence that serotonin (5-HT) plays any part in human allergy, indeed it seems that a major rôle is unlikely. Severe bronchospasm is not produced in man by an aerosol of 5-HT, nor by intravenous infusion, and human bronchiolar muscle in vitro does not contract to 5-HT".

Effect on blood vessels

GADDUM et al. (1953) reported that 5-hydroxytryptamine causes vasoconstriction and bronchoconstriction in the cat's lung. RODDIE et al. (1955), GLOVER et al. (1958), HADDY et al. (1962) reported that a vasoconstrictor effect is obtained with the intra-arterial administration of 5-hydroxytryptamine.

HARRIS et al. (1960) also reported that 5-hydroxytryptamine causes pulmonary hypertension by local vasoconstriction while AVIADO (1960) said that the main vasoconstrictor action of 5-hydroxytryptamine is of the arteries, followed by the vasoconstriction of small venules (0.4 mm) and large veins.

According to GINZEL and KOTTEGODA (1953) 5-hydroxytryptamine is a more potent pulmonary vasoconstrictor than adrenaline or noradrenaline, a fact which has been confirmed by other inves-

tigators [BORST et al. (1957), ATTINGER (1957, 1959), GILBERT et al. (1957, 1958), ROSE and LAZARO (1958), etc.].

GILBERT et al. (1958) studied the effects of histamine, 5-hydroxytryptamine and adrenaline on pulmonary hemodynamics and found that histamine usually caused a greater increase of the venous resistance than of the arterial resistance, while 5-hydroxytryptamine usually increased the arterial resistance more than the venous resistance.

REID (1952) reported that 5-hydroxytryptamine causes pulmonary vasocontriction following intravenous administration. He ascribed the rise in systematic arterial pressure to direct action on the vascular smooth muscle. REID stated that in subjects with clearly visible subcutaneous veins, 5-hydroxytryptamine caused a total disappearance of the venous network.

PAGE and MCCUBBIN (1953) concluded that arterial pressure response to 5-hydroxytryptamine is the resultant of several variables of which four are of major importance: (1) a direct vasoconstrictor action, (2) a Von-Bezold-Jarish-like reflex, (3) transient autonomic ganglion blockade and (4) peripheral inhibition of neurogenic vasoconstriction. Furthermore, according to BOCK et al. (1957) and LE MESSURIER et al. (1959), 5-hydroxytryptamine causes vasodilatation of skeletal muscles in man when given by infusion (BOCK et al. administered 0.1-100 γ; and LE MESSURIER et al. 1, 2 and 3 mg/min.).

In 1960, LEWIS reported that 5-hydroxytryptamine causes vasoconstriction of the pulmonary circulation with a rise in pulmonary artery pressure. In fact, this vasoconstrictor effect of 5-hydroxy-(tryptamine has been confirmed by HADDY et al. (1957, 1959), GLOVER et al. (1958), KABINS et al. (1960), GROLLMAN (1960), BRAUN and STERN (1961), GOTH (1961), VITOLO et al. (1962), BROCKLEHURST (1962), ALEXANDER (1963), etc..

VITOLO et al. (1962) confirmed the influence on the systemic arterial pressure. They studied the mechanism of pulmonary vasoconstriction after injection of 5-hydroxytryptamine. Their results showed that 5-hydroxytryptamine has a hypertensive effect on the pulmonary artery and vein, raising the arteriovenous gradient. Neither ganglionic block nor adrenalectomy modified this hypertensive effect on the vessels. These authors concluded that the pulmonary vasoconstriction provoked by 5-hydroxytryptamine is due, neither

to neurogenic mechanisms nor to humoral mechanisms, but to direct action on the pulmonary vessels. "Serotonin, which has captured physiological and clinical imaginations on many different accounts, is also generally held to be a uniquely effective pulmonary arterial and venous vasoconstrictor" (FISHMAN, 1963).

No report was found concerning the action of 5-hydroxytryptamine on the nasal blood vessels.

Effect on capillary permeability

PARRATT and WEST (1958) studied the relationship of 5-hydroxytryptamine to capillary permeability in the skin of the rat and concluded that 5-hydroxytryptamine, as well as histamine, plays a rôle in the production of increased capillary permeability, by histamine liberators (polymyxin and 48/80).

In 1961, MAJNO and PALADE reported their electron microscopic results regarding the effect of histamine and 5-hydroxytryptamine on the vascular permeability in the rat. These agents were injected subcutaneously into the scrotum. These authors stated: "Serotonin may be said to be roughly 100 times more active than histamine, on a mole-to-mole basis ... This was, in fact the only difference found in our study between the effects of the two drugs". They concluded " ... the mechanism which operates to increase the vascular permeability — the partial dissociation of the endothelial sheet — is, possibly, the simplest". The results of these investigators, strongly suggest the venular side as the site of action of histamine and 5-hydroxytryptamine.

SPECTOR (1958) reported that the "importance of serotonin as a 'mediator' of altered vascular permeability in inflammation is likely to be confined to certain tissues, perhaps only the skin of the rat and possibly of te mouse". HERXHEIMER and SCHACHTER (1959) said that the local administration of 5-hydroxytryptamine, in man, does not produce oedema. MELMON et al. (1965) reported a case (female, 58 years) who experienced attacks of "body flushing" (carcinoid syndrome) associated by dyspnoea, lacrimation, "nasal stuffiness", preorbital oedema, generalized pheripheral oedema, oliguria and complete debility. The incidence of transient swelling of the face and localized oedema, however, "is too low to be certain

that they are not coincidental . . ." (STACEY, 1966). GARATTINI and VALZELLI (1965) stated that there is insufficient evidence to assume that 5-hydroxytryptamine is physiologically involved in the regulation of the capillary permeability in man. "In fact, 5-hydroxytryptamine which, in high concentrations (100-2500 μg/ml), is an effective flaring agent when given intradermally in man, fails to cause whealing or other evidence of increased capillary permeability" (ERSPAMER, 1966).

Effect on eosinophils

VOORHORST (1963) stated that 5-hydroxytryptamine has chemotactically no influence on eosinophils.

ARCHER (1963) reported that changes in eosinophil concentration could not be observed with 5-hydroxytryptamine because it is not eosinophilotactic, nor does it destroy them in the conditions used. However, STEINER and HEDINGER (1956), ROSA et al. (1958) and STACEY (1966) reported that 5-hydroxytryptamine induces eosinopenia in animals and in man.

Effect on mucous glandular secretion

From the abundant literature concerning 5-hydroxytryptamine, no report was found about the action of 5-hydroxytryptamine on the secretion of the nasal glands.

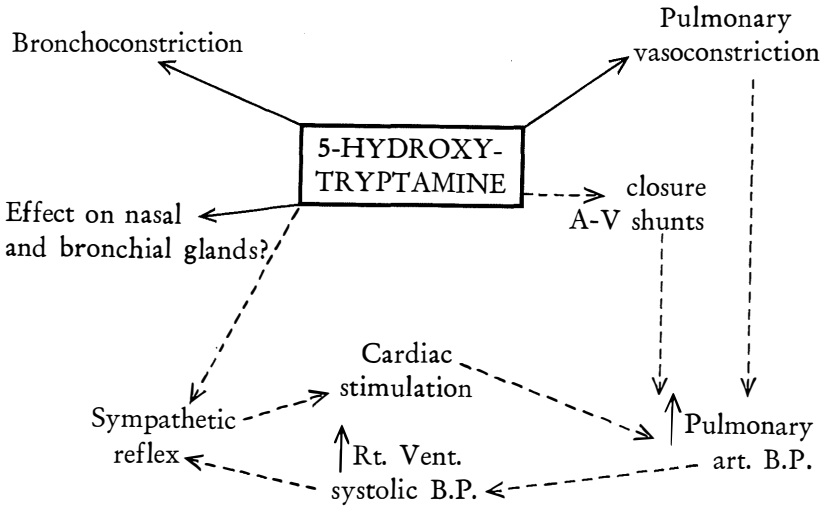


Fig. 13. Actions of 5-hydroxytryptamine
[After AVIADO, 1965 (slightly modified)].

Own clinical experiments

The effect of 5-hydroxytryptamine on the nasal respiratory mucosa was studied in 10 patients with nasal complaints. The histamine threshold was determined, the day before the determination of the effect of 5-hydroxytryptamine on the nasal respiratory mucosa. All experiments were carried out exactly in the same way as described before (chapter III). The following concentrations of 5-hydroxytryptamine (as the creatinine sulphate salt) were applied: 0.1, 1, 2* mg/ml. The duration of each 5-hydroxytryptamine application was 5 min..

The results obtained are shown in table 48.

Table 48

Comparison of the effect of histamine and 5-hydroxytryptamine on the nasal respiratory mucosa by means of topical application, in patients with nasal complaints.

No.	Histamine reactivity expressed as x of 2^x mg/ml	P_I in cmH_2O	Pharynx-nostril pressure gradient in cmH_2O after 5-hydroxytryptamine application	Observation					
				Histamine			5-hydroxytryptamine		
				O	N	S	O	N	S
1	—2	4.2	5.6	+++	—	+	—	—	—
2	—1	9.8	5.6	+++	—	—	—	—	—
3	—2	7.0	8.4	+++	—	+	—	—	—
4	0	7.0	7.0	+++	—	+	—	—	—
5	2	2.8	2.8	++	—	+	—	—	—
6	—2	4.5	5.6	+++	—	—	—	—	—
7	0	5.6	5.6	+++	—	+	—	—	—
8	—1	2.8	2.8	++	—	+	—	—	—
9	—2	8.4	8.4	+++	—	+	—	—	—
10	0	4.2	4.2	++	—	+	—	—	—

O : Swelling of the mucosa
N : Sneezing
S : Hypersecretion
P_I : Initial pressure value
— : No reaction
\+ : Slight
++ : Moderate
+++ : Severe

In another trial the response of the nasal respiratory mucosa and the bronchial tree was assessed separately to 0.125, 0.25, 1.5, 1 and 2 % 5-hydroxytryptamine (creatinine sulphate salt) in 14 patients. The same technique of application and assessment of the response of the nasal mucosa were used as described before. The

* 0.0049 Mol/lit.

Table 49

A comparison between the response of the nasal respiratory mucosa, and the bronchial tree to 5-Hydroxytryptamine in 14 patients with nasal and chest complaints.

No.	Age	Sex	N_{HR}	B_{HR}	Predicted value		Measured value		VC and FEV_1 in % of predicted value		Initial pharynx-nostril pressure gradient in cmH_2O	Pharynx-nostril pressure gradient in cmH_2O after the application of 2 % 5-hydroxytryptamine (creatinine sulphate)	Bronchial 5-hydroxytryptamine (creatinine sulphate) threshold value
					VC	FEV_1	VC	FEV_1	VC	FEV_1			
1	19	F	3	—2	4220	3249	2525	1300	77	52	2.1	4.2	0.25 %
2	16	M	1	—2	3600	2880	2950	1625	82	56	3.5	3.5	0.25 %
3	19	M	4	—2	5200	4004	4150	3400	80	85	2.1	1.4	> 2 %
4	21	F	0	—1	3940	3011	4025	3200	102	106	1.4	1.4	> 2 %
5	16	M	4	—3	2830	2264	2425	1987	86	88	9.0	9.2	> 2 %
6	17	M	—1	—3	4130	3304	2975	2075	72	63	3.5	3.5	> 2 %
7	17	M	—1	—1	5040	3880	2875	2425	57	63	7.6	7.3	> 2 %
8	14	M	—1	—3	4270	3416	3325	2500	78	73	7.0	6.0	0.125 %
9	54	M	2	1	3410	2217	2150	650	63	29	7.7	7.0	> 2 %
10	45	M	1	—2	3920	2705	3775	1650	96	61	2.1	2.5	2 %
11	50	M	2	—1	4750	3088	4250	2450	90	79	8.4	5.6	2 %
12	48	M	2	—2	3920	2548	2500	825	64	32	8.8	8.8	> 2 %
13	57	M	0	—1	4180	2717	4150	2500	99	92	6.3	6.3	> 2 %
14	49	M	3	0	4610	2997	2950	1425	64	48	7.7	7.7	> 2 %

N_{HR} : Nasal histamine reactivity)
B_{HR} : Bronchial histamine reactivity) expressed as x of 2^x mg/ml
VC : Vital Capacity
FEV_1 : Forced Expiratory Volume in one second

method used for the assessment of the bronchial response to nebulized 5-hydroxytryptamine, is the same as has been described for histamine (see chapter III, § 6), except that 5-hydroxytryptamine was inhaled for 3 min.. The results are summarized in table 49.

Conclusion

5-Hydroxytryptamine *had no effect* on the nasal respiratory mucosa when applied in concentrations of 2 mg/ml or 2 % by means of direct topical application, while inhalation of 5-hydroxytryptamine *caused severe bronchial obstruction.*

§ 4. Bradykinin

Bradykinin was discovered by ROCHA E SILVA et al. (1949) in 1948 during studies on the anticoagulating properties of snake venom. It has been isolated by ELLIOTT et al. (1960) and synthetized by BOISSONNAS et al. (1960). Bradykinin is present in normal blood as an inactive precursor viz. bradykinogen, a component of the α2-globulin fraction of the plasma from which it is released by proteolytic enzymes (e.g. snake venoms and trypsin) and is regarded as a potent pharmacological agent (ROCHA E SILVA et al. 1949). "It has now been established that bradykinin is identical with or closely related to the active product formed by the action of kallikrein on plasma proteins, which is called kallidin" (LEWIS, 1960).

SHORLEY and COLLIER (1960) compared the synthetic nonapeptide (L-arginyl-L-propyl-L-glycyl-L-phenylalanyl-L-seryl-L-propyl-L-phenylalanyl-L-arginine) with trypsin bradykinin: "On the guinea-pig lungs in vivo, both nonapeptide and bradykinin caused bronchoconstriction". In fact, KONZETT and STÜRMER (1960) and LEWIS (1960) also found the synthetic nonapeptide (H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH) identical with trypsin bradykinin. BURCH and DEPASQUALE (1963) said that all of the substances (plasma kinins) studied thus far which have these properties (stimulation of smooth muscle, vasodilatation), are virtually identical to bradykinin, if not the same substance. From the literature, the following pharmacological properties could be attributed to bradykinin: stimulation of smooth muscle, vasodilatation, increase in capillary permeability, stimulation of pain fibres and migration of leukocytes.

Effect on smooth muscle

ROCHA E SILVA et al. (1949) reported that bradykinin has been found to stimulate all smooth muscle structures tested. ELLIOTT et al. (1960) confirmed this finding on isolated smooth muscle preparations (guinea-pig ileum, rat uterus, rat duodenum and rabbit duodenum).

WAALER (1961) studied the effect of bradykinin in an isolated perfused dog lung preparation and stated: "the airways of the isolated perfused dog lung preparation were never constricted and in some experiments were slightly dilated by bradykinin". WAALER found either a small bronchodilatation or no effect on the tidal air on injections [0.9 g/100 ml (0.1-1.0 ml)] of pure bradykinin into the pulmonary artery.

On the other hand, BISSETT and LEWIS (1962) found bradykinin as the most active peptide in causing bronchoconstriction in the intact animal which was different from that produced by histamine. The increase in bronchiolar tone was more gradual in onset and of longer duration than in the case of histamine. COLLIER et al. (1960) demonstrated bronchoconstriction in the guinea-pig in doses ranging from 1.25 to 80 units — doses of 2.5 to 10 units being effective (± 12,000-12,500 units were equivalent to 1 mg pure bradykinin). This action these investigators found, was not affected by vagotomy or by treatment of the animal with mepyramine, atropine, lysergic acid diethylamide, or cortisone. They also found that the closely related peptide, wasp kinin, was a potent bronchoconstrictor. BERRY et al. (1963) reported that injection of 1-2 µg synthetic bradykinin did increase the resistance of the lung of a guinea-pig.

BH●●LA et al. (1962), however, reported that bradykinin did not contract isolated dog bronchus (15 µg/ml), nor 4 preparations of human bronchus (up to 667 µg/ml) which responded to histamine (0.067 tot 0.67 µg/ml) and acetylcholine (0.2 to 0.67 µg/ml).

HERXHEIMER and STRESEMANN (1961) studied the effect of bradykinin aerosol in guinea-pigs and in man and found that bradykinin in concentrations of 0.5, 1 and 2 % did not cause any dyspnoea in the guinea-pig while severe dyspnoea occurred with 0.25 % acetylcholine, 0.5 % histamine and 1 % 5-hydroxytryptamine. Intravenous injection, however, caused bronchospasm. These investigators further found that a 0.5 % bradykinin aerosol was very active in asthmatic patients — 13 patients (total 15) showed a decrease of the vital

capacity of 10-30 % below normal, accompanied by audible wheezing and coughing. In normal individuals no reaction was noted. STRESEMANN (1963) also studied the effect of bradykinin in asthmatic patients by aerosol (0.5 %) and observed bronchospasm.

LECOMTE et al. (1962) studied the effect of bradykinin (0.1 % and 0.8 % administered per aerosol) in normals and in asthmatics. On the administration of 0.1 % bradykinin, these investigators found no reaction in normals and minor changes of the lung volumes in the asthmatics. However, with 0.8 %, LECOMTE et al. found profound changes of the lung volumes in the asthmatics and concluded: "La bradykinine possède des proprietés broncho-constrictrices importantes chez l'homme asthmatique".

Effect on blood vessels

ROCHA E SILVA et al. (1949) reported that bradykinin when injected in doses of 5 to 10 mg, produces a shock-like condition, with a steady fall in arterial blood pressure. BISSETT and LEWIS (1962) also reported that bradykinin is a vasodilator. WAALER (1961) said: "In the present experiments on an isolated perfused dog lung preparation, bradykinin caused vasodilation of the pulmonary vascular bed, which, however, seemed to be less sensitive than other vascular fields".

FOX et al. (1961) reported experiments performed on 19 healthy adult males. These investigators found bradykinin a potent vasodilator: "On intravenous administration the first effect of threshold doses of bradykinin (0.1-0.4 μg/kg) was flushing of the face and neck. With larger doses this vasodilatation became intense and spread to the rest of the body. Histamine was more active than bradykinin by the intravenous route". BISHOP et al. (1962) studied the effect of synthetic bradykinin on the pulmonary and systemic circulation in 7 normal subjects, and 7 patients suffering from chronic bronchitis and emphysema. A constant infusion of synthetic bradykinin, in a dose ranging from 0.2 to 1.0 μg/kg/min. for 15 min. was administered either into a cubital vein or the superior vena cava. An increase in cardiac output associated with an increased heart rate and stroke volume was noted in all normal and all, but one of the patients; "there was usually no evidence of a

change in pulmonary vascular resistance". These investigators did not observe bronchoconstriction and concluded that the pulmonary vascular bed responds by dilatation only at the higher dosage. They suggested that small doses may sometimes cause pulmonary vasoconstriction at the same time as systemic vasodilatation.

"Bradykinin administered intra-arterially or intravenously to man is a potent vasodilator substance" (Fox et al., 1960). ALLWOOD and LEWIS (1963) studied bradykinin in human blood during vasodilatation but did not find an increase of bradykinin in blood samples during vasodilatation, however, these authors said, this fact does not exclude bradykinin as a mediator of the dilatation.

DE FREITAS et al. (1964) administered bradykinin intravenously (2.5 mg/100 ml) to study the general circulatory alterations in man, submitted to simultanous right and left heart catheterization. Ten patients were studied. These investigators found a significant drop in systemic arterial pressure and in total peripheral resistance accompanied by an increase of the cardiac output, heart rate and stroke volume. "There was no consistent change of the pulmonary arterial and left atrial pressures, but the pulmonary vascular resistance decreased significantly". Bradykinin was also given intravenouly, 25 μg/ml of saline by KONTOS et al. (1964) in studying the general and regional circulatory effects of bradykinin in man. These authors stated that bradykinin is a powerful vasodilator of cutaneous blood vessels in the human being and concluded "comparable doses injected into the superior vena cava or pulmonary artery increased pulmonary arterial pressure".

"As a vasodilator both in animals and man bradykinin is one of the most potent substances known. . . ." (LEWIS, 1963).

Effect on capillary permeability

The effect of increased capillary permeability by bradykinin has been reported by different authors, [e.g. HOLDSTOCK et al. (1957), HERXHEIMER and SCHACHTER (1959), COLLIER et al. (1960)]. ELLIOTT et al. (1960) stated: "On a molar basis bradykinin was approximately 15 times more active in increasing permeability than histamine". SCHACHTER (1960) and ELLIOTT et al. (1961), found bradykinin on a molar base, about 10 times more active than histamine in increasing

capillary permeability. SHIMAMOTO (1964) suggested B_{23} or nialamide or aminopyrine as antagonists to the "vascular permeability-increasing activity" of bradykinin.

"Our results demonstrate that bradykinin (and hence kallidin which appears to be identical with it) is one of the most effective substances in increasing capillary permeability" (SCHACHTER, 1963).

Effect on mucous glandular secretion

From the literature no evidence exists that bradykinin is a mucous glandular stimulant, as in the case of histamine or acetylcholine. Bradykinin causes vasodilation in the salivary gland upon stimulation of the chorda tympani — atropine does not suppress this effect [HILTON and LEWIS (1956) and BURCH and DEPASQUALE (1963)].

Effect on eosinophilic leukocytes

No study has shown yet that bradykinin has any chemotactic effect on eosinophils. In fact, ARCHER and BR●●ME (1963) reported that bradykinin is not chemotactic for eosinophils.

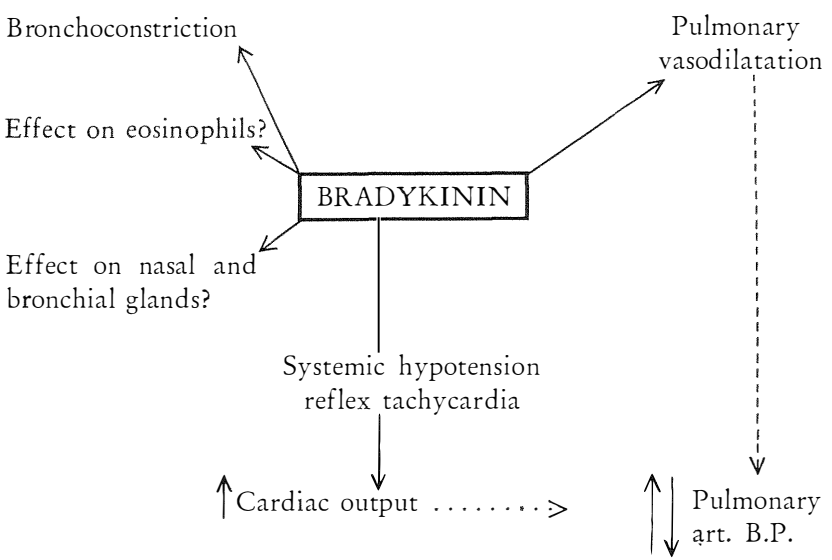


Fig. 14 Actions of Bradykinin
[After AVIAD●, 1965 (slightly modified)].

Table 50

Comparative results - showing the effect of histamine, bradykinin, kallikrein and padutin® on the nasal respiratory mucosa by means of topical application, in patients with nasal and chest complaints.

No.	P_I in cm H_2O	Histamine reactivity expressed as x of 2^x mg/ml	Observation			Pharynx-nostril pressure gradient in cmH_2O after application of			Observation		
			O	N	S	Bradykinin	Kallikrein	*Padutin®	O	N	S
1	5.6	0	++	—	+	5.6	4.8	5.6	—	—	—
2	4.2	—2	+++	+	++	4.5	4.3	4.5	—	—	—
3	4.2	—1	+++	—	—	4.2	4.2	4.2	—	—	—
4	2.8	—1	+++	—	+	3.4	4.2	4.2	—	—	—
5	9.1	0	++	—	—	10.6	10.6	10.6	—	—	—
6	5.6	—2	+++	—	+	4.2	4.5	4.5	—	—	—
7	3.5	—1	+++	—	+	3.5	3.5	3.5	—	—	—
8	4.5	4	++	—	+	4.6	4.7	4.5	—	—	—
9	7.5	—1	+++	—	—	7.0	7.3	7.4	—	—	—
10	6.5	—1	+++	—	++	6.5	6.3	6.3	—	—	—
11	5.6	6	±	—	—	5.6	5.6	5.6	—	—	—
12	4.3	2	++	—	+	4.4	4.5	4.5	—	—	—
13	3.8	0	+++	—	+	4.0	4.2	4.0	—	—	—
14	6.5	—2	+++	+	++	6.3	6.5	6.6	—	—	—
15	4.5	—4	+++	+	++	4.5	4.5	4.5	—	—	—

O : Swelling of the mucosa
N : Sneezing
S : Hypersecretion
P_I : Initial pressure value
— : no reaction
\+ : Slight
++ : Moderate
+++ : Severe

*: pancreatic kallikrein (padutin®)

Table 51

Some simple lung function data of the patients with nasal and chest complaints in whom the effect of bradykinin, kallikrein, pancreatic kallikrein (padutin®) and histamine was compared by means of topical application to the nasal respiratory mucosa.

No.	Sex	Age	B_{HR}	Predicted value		Measured value		VC and FEV_1 in % of predicted value	
				VC	FEV_1	VC	FEV_1	VC	FEV_1
1	F	34	6	3390	2475	2975	2850	88	115
2	M	39	3	5400	4200	5700	3850	106	92
3	M	26	4	3950	3050	3300	2900	84	95
4	M	19	0	4720	3634	4325	2700	92	74
5	M	17	5	4270	3500	4300	3550	101	101
6	M	35	4	4925	3595	4950	3600	101	100
7	M	25	2	4150	3180	3950	2800	95	88
8	F	18	3	4350	3400	4150	3150	95	93
9	F	27	6	4700	3572	4475	3825	95	107
10	F	21	2	3850	2870	3650	2575	95	90
11	F	28	4	4480	3440	4500	3050	100	89
12	M	60	2	4165	2707	3800	2300	91	85
13	M	54	3	4250	3550	3850	2575	91	73
14	M	40	1	4250	2950	4250	2550	100	86
15	M	21	5	4840	3720	5125	3650	106	98

B_{HR} : Bronchial histamine reactivity expressed as x of 2^x mg/ml
VC : Vital Capacity
FEV_1 : Forced Expiratory Volume in one second

Miscellaneous

Bradykinin brought into contact with nerve endings — causes pain [ARMSTRONG et al. (1954, 1957), ELLIOTT et al. (1960), LEWIS (1960), etc.].

Own clinical experiments

To evaluate the effect of bradykinin on the nasal respiratory mucosa, 15 patients with nasal and chest complaints were selected. Since kallikrein and pancreatic kallikrein (padutin®) were available (and unexpensive!) these substances were included in this evaluation. The application of bradykinin, kallikrein and padutin® was performed and judged in exactly the same way as has been described for histamine (see chapter III). First, the histamine reactivity was established, afterwards on another day, the effect of bradykinin, kallikrein and padutin® was examined. Bradykinin was applied for as long as 6-10 min. using the concentrations: 0.001, 0.01 and 0.1 %. If no reaction or any changes occurred, kallikrein 1 unit(u.),

10u.) was applied to the nasal respiratory mucosa for 5 min.. If no reaction occurred after the application of kallikrein (10u.), padutin® (20u. and 40u.) was applied to the nasal respiratory mucosa for 5 min..

The results are shown in table 50.

Conclusion

Bradykinin, kallikrein (and padutin®) applied to the nasal respiratory mucosa in concentrations as described above, *had no effect on the nasal respiratory mucosa.*

§ 5. Histamine-releasing agents

General introduction

Histamine is widely distributed throughout the body in many tissues and inside these tissues in certain cells [FELDBERG (1956), GADDUM (1956), etc.]. The rôle, however, it plays in the functions of the cells in which it resides, is yet not fully understood. "Histamine has such pronounced pharmacologic actions, that its presence in quantities in excess of these required for physiologic processes will quickly produce abnormalities. These are often the symptoms of disease" (CODE et al., 1964).

Different types of histamine-releasing agents

Many agents so far studied, possess the general property of releasing histamine but chemically they are heterogeneous and according to HALPERN: "the action of the various histamine releasors differs, however, according to their chemical structure", e.g. in the dog, compound 48/80 releases histamine mainly from the liver and muscles, tween-20, polyvidone and sinomenine, however, liberate histamine mainly from the skin and to a lesser extent from the liver and muscle (NISHIYAMA et al., 1937).

Few examples of histamine-releasing agents are:

Antihistaminics [ARUNLAKSHANA (1953), HÖGBERG and UVNÄS (1957), SMITH (1958), MOTA and DIAS DA SILVA (1960), etc.].

Morphine [SALTER and WHITE (1949), NASMYTH and STEWART (1950), MCINTIRE et al. (1951), MONGAR (1956), PARRATT and WEST (1957), etc.].

Atropine, Codeine and Thebaine [FELDBERG and PATON (1951), etc.].

Nalorphine [PATON (1957), etc.].

Papaverine [Feldberg and Paton (1951), etc.].
Reserpine [Waalkes et al. (1959), etc.].
Quinine, Meperidine (pethidine), Tolazoline (priscol) and Neoarsphenamine [Schachter (1952), etc.].
5-Hydroxytryptamine and Tryptamine [Feldberg and Smith (1953), etc.].
d-Tubocurarine [Rocha e Silva and Schild (1949), Mongar and Schild (1952), Mongar and Whelan (1953), etc.].
Horse serum [Feldberg and Schachter (1952), etc.].
Trypsin [Rocha e Silva (1944), etc.].
Ammonia [Schild (1949), etc.].
Adrenaline [Eichler and Barfuss (1940), Koch and Szerb (1950), Szilágyi et al. (1960), etc.].
Snake poisons [Feldberg and Kellaway (1937), Dutta and Narayanan (1952), etc.].
Stilbamidine [McIntosch and Paton (1949), etc.].
Peptone [Feldberg and O'Connor (1937), etc.].
Polymyxin B [Norton and de Beer (1955), Parratt and West (1957), etc.].
Toluidine blue [Smith (1958), etc.].
Compound 1935-L [Feldberg and Lecomte (1955), Mélon and Lecomte (1958), Halpern (1960), etc.].

Site of action of histamine release

The above examples of histamine-releasing agents raise the question of the site of action of these substances. The precise mechanism, however, of histamine release by exogenous stimuli remains uncertain [Paton (1956, 1957), Spector (1958), etc.].

Histamine is widely distributed throughout the body and its distribution varies in different species (Feldberg, 1956). According to Halpern (1953) the histamine present in the body can be said to exist in three forms: combined, labile and free. Spector and Willoughby (1965) suggested that combined histamine is released only by cellular destruction, and the labile histamine by means of chemical and physical stimuli. "There are usually only traces of free histamine".

The cells which liberate histamine are believed to be those richest in it, viz. the *basophils of the blood* [Valentine et al. (1955), Gra-

HAM et al. (1955), CODE and MITCHELL (1957), LECOMTE and BAECKELAND (1963), etc.], and the *mast cells of the tissue* [RILEY and WEST (1953), RILEY (1956), WEST (1956), BENDITT et al. (1956), SJOERDSMA et al. (1957), BLOOM (1965), etc.]. Of these, the mast cells are presumably the most important (LECOMTE and BAECKELAND, 1963). Variations in tissue histamine run parallel to morphological changes in the mast cells [RILEY and WEST (1955), etc.].

MOTA et al. (1953) and MOTA and VUGMAN (1956) studied the mast cell reaction in rats and guinea-pigs and found that the histamine content of the lung (guinea-pig) is closely related to its mast cell population. When isolated mast cells from a sensitized animal are exposed to a specific antigen, degranulation of these cells occurs with release of histamine [e.g. UVNÄS and THON (1959), MOTA and DIAS DA SILVA (1960)]. Furthermore it has been shown that the mast cells are capable of actively releasing histamine without degranulation (SMITH, 1958). HÖGBERG and UVNÄS (UVNÄS, 1958) carried out experiments on incubated rat mast cells in vitro and found that 48/80 and a tertiary amine related to 48/80 released histamine from these cells. By means of certain enzyme inhibitors, e.g. 1.3-diphosphoimidazole (DPI) and by prior heating of the mast cells to 50° C, the release of histamine from these cells could be prevented. These authors concluded that a lytic enzyme with an essen-

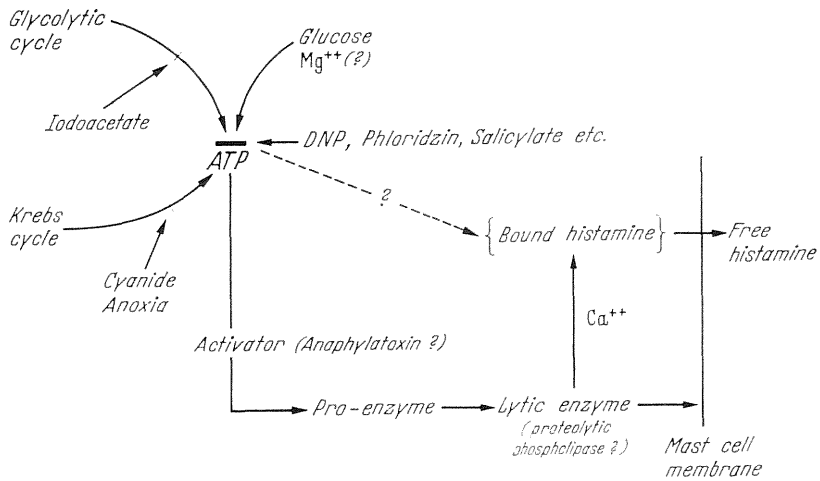


Fig. 15

Possible chain of events in the mechanism of histamine release, showing enzymes of the carbohydrate metabolism and activation of a lytic enzyme which might be responsible for the breakdown of the mast cell membrane.
(According to Rocha e Silva, 1960).

tial amino group presumably exists on the surface of the mast cells.

Despite difficulties in the integration of these observed experimental facts, SPECTOR and WILLOUGHBY (1965) stated that: "It is now generally agreed that histamine can be released by at least four mechanisms: the splitting of a hypothetical bond linking histamine to a protein; leakage of histamine through a cellular or subcellular membrane rendered permeable or destroyed; an ion exchange reaction releasing histamine from combination with an acidic intracellular site; or rupture of a polar linkage with protein or lipoprotein".

Histamine release in normal and allergic invididuals

"Allergic manifestations, such as the production of transient asthma, urticaria, changes in the nasal mucous membrane or production of headaches, could be produced only in those patients who actively suffered from such complaints" (ROSE, 1947). KATZ and COHEN (1941) demonstrated that in such patients, histamine shifts from the cells to the plasma.

SCHILD et al. (1951) showed that histamine is released from the bronchioles and lung tissues in asthmatic patients when, a specific antigen to which the patient is sensitive, is added to an isolated portion. They showed vigorous contraction of chains of bronchial rings, suspended in Ringer's solution following addition of the specific allergen. The bronchial rings were also sensitive to histamine and acetylcholine. Bronchial tissue from normal persons did not respond. This raises the possibility of an altered reactivity. ". . . We must accept as a fact that the allergen-antibody reaction leads to liberation of histamine (and other substances) in man" [LINDELL and WESTLING, 1966].

ROSE (1954) advocated the fact that in subjects with allergic disease — there must be a shift of histamine from the intracellular or inactive form to the extracellular or free state (pharmacologically active form).

"The relationship between release of histamine and clinical allergy is stressed by the facts that, in allergic individuals, the injection of a chemical histamineliberator (1935-L) produces the usual spontaneous clinical symptoms (urticaria, asthma). . ." (HALPERN, 1960).

More recently, KAHLSON et al. (1966) reported that the tissues of individuals suffering from "hypersensitive states", are exposed to

histamine which is generated in two ways with different time courses, namely "an initial very brief phase of release of histamine, and a supervening prolonged phase of new formation" ("nascent" histamine). The effects of the latter are not abolished by antihistaminics, nor can such effects be reproduced by exogenous histamine. The "nascent" histamine KAHLSON et al. said, is not firmly bound at the sites of its formation and it is believed to be largely of non-mast cell origin.

"There is little reason to doubt that in man, as in other species studied, histamine release is accompanied by elevations of H.F.C.* in various tissues including the lung, which is known to form histamine ..." and this "nascent" histamine, "... may at least partly account for manifestations which formerly were not believed to be associated with histamine" (KAHLSON et al., 1966).

HALPERN (1960) studied the response of histamine liberators in normal and allergic subjects, and found in normals (non-allergic subjects) on injection of 0.1 mg/kg, the same clinical symptoms produced by histamine: erythema, generalized prickling, pulsatile headache, fall of blood pressure, and gastric hypersecretion and controlled these symptoms with antihistaminic drugs. In allergic individuals, he found two phases: (a) symptoms identical with those in non-allergic individuals; (b) a second phase during which the clinical symptoms are exactly reproduced. HALPERN found in these allergic individuals a very interesting characteristic, viz.: repeated injections [with histamine liberators (1935-L)] at adequate intervals of time, causes mounting resistance to the histamine-liberator, which is accompanied by a considerable clinical improvement, in the allergic symptoms.

Replenishment of histamine

Histamine is formed at its storage sites by decarboxylation of L-histidine [MELLANBY and TWORT (1912), BLOCH and PINÖSCH (1936), GADDUM (1956), SCHAYER (1956, 1966), SICE (1962), LINDELL and WESTLING (1966), etc.].

Animal experiments indicate that adrenocortical hormones (cortisone) influence the replenishment of histamine (decreased rate of formation) [SCHAYER et al. (1955), LECOMTE (1955), SCHAYER (1956), HALPERN (1956, 1960), TELFORD and WEST (1960, 1961), etc.].

* H.F.C.: Histamine Forming Capacity.

Restoration of histamine varies after depletion by a histamine releasor, e.g. FELDBERG and TALESNIK (1953) stated "... injections of 500 μg 48/80 into the saphenous artery of dogs produce, ... pronounced reduction in the histamine content of the skin ... the histamine content of the skin remains low for weeks", while HALPERN (1956) reported that the skin histamine content of rats after depletion by dextran, was 27 ± 8.7 μg/kg (after 5 hours) and 63 ± 20.7 μg/kg (after 120 hours); the histamine content of the skin in control animals was 79 ± 25 μg/kg. However, HALPERN (1960) stated that when cortisone is injected into animals whose histamine has been depleted by treatment of a histamine-releasing agent, the replenishment of histamine is considerably slowed.

TELFORD and WEST (1960) showed that cortisone can cause morphological changes in the mast cells.

Some experiments in man producing decrease in blood histamine [CODE and MITCHELL (1957), NOAH and BRAND (1957), etc.] and the concomitant increase in the urinary histamine content [MITCHELL and CODE (1954), MITCHELL et al. (1954), etc.] after the administration of cortisone in normals and allergic individuals and, the clinical experience that — "... cortisone is definitely effective in various clinical allergic syndromes conditioned apparently by liberation of histamine: hay fever, asthma ..." (HALPERN, 1956) — suggest that cortisone (corticosteroids) may influence, also in man, the replenishment of histamine. The stage at which cortisone acts in the "cycle of replenishment" of histamine however, remains unknown (HALPERN, 1960).

Other hormones are apparently also of importance in histamine restoration: "the high content of histamine in the skin, in thyreotoxicosis, and the reduced values in myxoedema ..., also point to an effect of the thyroid hormones on histamine metabolism" (LINDELL and WESTLING, 1966).

Simultaneous release of other substances

HALPERN (1956) studied the plasma histamine content in rats following the intravenous injection of 180 mg dextran and found a profound release of histamine. However, in samples of plasma withdrawn 5 min. following the injection of dextran (at the moment of shock and prostration of the animals) - he found "besides hista-

mine another substance, which produces a much slower contraction than histamine and which seems to be identical with the so-called 'slow reacting substance' ". PATON (1951) also found, beside histamine release, a slow reacting substance in plasma of the cat and dog, after the injection of compound 48/80.

In 1957, PARRATT and WEST reported that 48/80 releases maximal amounts of histamine in the rat - "but it is a much more active releasor of 5-HT, whereas the reverse is true for polymyxin B. Since 5-HT, unlike histamine, does not occur mainly in mast cells, at least in skin, it is not surprising that substances like compound 48/80 and polymyxin B, which release both amines, differ quantitatively in their effects".

Allergic and non-allergic animals

DALE (1913) demonstrated that isolated sensitized animal (guinea-pig) smooth muscle could react to an antigen and that normal smooth muscle could passively be sensitized with serum from a sensitive animal. More recently, PATTERSON et al. (1963) demonstrated reagenic antibodies against "ragweed pollen" in dogs. LOWELL (1964) observed an asthma-like syndrome in the horse. ARBESMAN et al. (1964) showed that monkeys can be passively sensitized with human reagins.

In 1952, MONGAR and SCHILD found a release of histamine in sensitized guinea-pigs on administration of an antigen (egg albumin) and by compound 48/80 and tubocurarine. However, "... with normal tissues, there is of course no release by the antigen, but 48/80 releases amounts comparable to those with sensitized tissues". These authors found that the histamine released by either antigen or synthetic liberators varies markedly in different tissues. SCHACHTER and TALESNIK (1952) proved that egg-white releases histamine from the isolated, perfused skin of sensitized cats; intravenous injection raises the plasma histamine level. However, in skin preparations of non-sensitized dogs, egg-white does not release histamine, nor does it raise the plasma concentration of histamine after intravenous injection.

Quantitative release in different tissues of various species

FELDBERG and PATON (1951) studied the release of histamine from skin and muscle in the cat and stated: "it was found that the histamine liberators liberate per gram skin up to between 15 and 30 μg of histamine, but per gram muscle only between 0.1 and 0.3 μg. This difference is due partly to the higher content of histamine in skin than in muscle, but also to the fact that most of the muscle histamine, unlike that of the skin, is resistant to the action of the histamine liberators".

FELDBERG and MONGAR (1954) compared the release of histamine by compound 48/80 and octylamine in perfused tissues of the hind legs and gastrocnemius muscle of the cat, the hind quarters of rats and guinea-pigs and lungs of all three species. On skin flaps and gastrocnemius muscle of the cat, these investigators found 48/80 about 200 times more active than octylamine, in the rat (perfused hind quarters preparation) 1000 times more active than octylamine and in the guinea-pig they found 48/80, 60 times more active than octylamine. To obtain these results only a few μg and on some preparations only a fraction of a μg of 48/80 was necessary; however, in the lungs perfused, doses of the order of 1 mg are necessary to release measurable amounts of histamine. Sligthly different results were obtained by MONGAR (1956) who compared the release of histamine by octylamine, morphine, d-tubocurarine and compound 48/80 from perfused tissues of the abovementioned animal species and gave the following results:

Table 52

Approximate threshold doses (mg), for histamine release by octylamine, morphine, d-tubocurarine and 48/80 from perfused tissues of different animal species.

	Octylamine	Morphine	d-tubocurarine	48/80
Guinea-pig lung	0.7	20	3.5	0.5
Guinea-pig limbs	1.0	3	0.2	0.05
Rat lung	0.3	3	1.1	0.2
Rat limbs	1.1	3	0.1	0.001
Cat lung	1.0	—	0.7	0.1

(According to Mongar, 1956).

". . . The threshold dose of a releasor depends on the tissue, species and method used . . ." (MONGAR, 1956).

"Although a relative dependence on the species is shown by some

basic releasors, it can be stated that in general, a basic compound, active as a histamine releasor in one species will also show effects in another, although often only at considerably different dosages or concentrations. Thus, compound 48/80 which is active in the tissues of the cat (PATON, 1951) and of the rat (FELDBERG and TALESNIK 1953) at the microgram level, will only act on the tissues of the guinea-pig in *vitro* at concentrations of 100 μg to 1 mg/ml and in *vivo* in doses of 10 mg/kg (SMITH, 1958). Amounts of compound 48/80, 200 to 400 times greater than those required to release 80 to 90 per cent of the histamine contained in isolated rat mast cells (UVNÄS and THON, 1959) are wholly ineffective when tested on rabbit platelet suspensions (HUMPHREY and JAQUES, 1955) even though the abundant histamine stores of these particles are easily mobilized by other basic releasors like morphine and octadecylamine (MCINTIRE et al. 1951).... Organ and tissue specificity are another feature of the action of basic histamine releasors" (ROTHSCHILD, 1966).

Histamine release and anaphylactic shock

"There can be no doubt that histamine plays a major rôle in the phenomenon of anaphylaxis in most animal species" (ROSE, 1947).

In 1932, DRAGSTEDT and GEBAUER-FUELNEGG demonstrated large amounts of histamine release in the dog when rendered anaphylactic and in 1941, ROSE found a correlation between the decrease in blood histamine and the severity of anaphylactic shock in the rabbit.

LECOMTE and BAECKELAND (1963) pointed out that the histamine responsible for the manifestation of cardio-vascular shock conditions, resides in the mast cells. "Presently, due to the many experimental data so far produced there is little room for doubt that most - although certainly not all - of the histamine released by antigen from sensitized tissues, comes from the mast cells in most of the species" (MOTA, 1966).

MONGAR and SCHILD (1952) compared the effects of anaphylactic shock and of chemical histamine releasors. They tried to see whether the quantities of histamine released were related to the histamine content of tissues and whether there was a parallelism between histamine released in anaphylaxis and by chemical releasors. For this

comparison they used adjacent pieces of tissue of similar thickness and structure from the same animal (guinea-pig). Chemical releasors (48/80 and d-tubocurarine) were added to the one and the antigen to the other. Sensitized guinea-pigs were obtained by injecting 100 mg of commercial egg albumin intraperitoneally and 100 mg subcutaneously, 3-6 weeks before the experiments. As antigen they used egg albumin. These investigators found a parallelism between percentage histamine released in the anaphylactic reaction and by the two chemical releasors: 48/80 and d-tubocurarine.

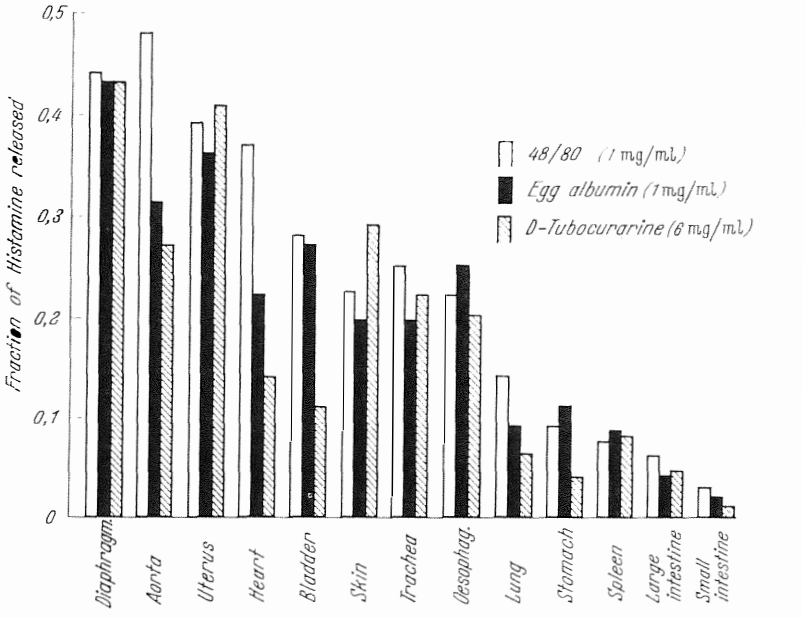


Fig. 16
Parallelism of histamine release by antigen and chemical releasors from guinea-pig tissues.
(According to Mongar and Schild, 1952).

Mota (1966) reported that the histamine content and the mast cell population of the guinea-pig lung is strongly correlated. In the lung of the guinea-pig rendered anaphylactic - small numbers of mast cells have been found by Mota and Vugman (1956). Mota (1966) stated that on administration of 48/80 - damage to the mast

cells occurs with reduction in the number of these cells. "As with antigen in anaphylaxis, this reduction is proportional to the concentration of the substance". MOTA (1966) concluded that the "anaphylactic phenomenon can be considered as a sequence of reactions starting by the union of antigen with antibody and having as one of its many consequences the release of histamine. In this respect it is closely related with the response of sensitized mast cells to antigen in most of the species".

Effect on the nasal mucosa

MÉLON and LECOMTE (1958), MÉLON (1963) studied the actions of histamine and of a histamine liberator on the nasal mucosa of allergic subjects, and found a vigorous reaction of the nasal mucosa to histamine, while the effect of the histamine liberator (1935-L) on the nasal mucosa was negative.

Compound 48/80

This compound is known today as one of the most active histamine releasors in animal species. It is a condensation product of paramethoxyphenethyl-methylamine with formaldehyde [BALTZLY, BUCK, DE BEER and WEBB (1949), etc.]. PATON (1951), FELDBERG and PATON (1951) and FELDBERG and SCHACHTER (1952), were the first to demonstrate that the vasodepressor activity of this compound, was due to the strong histamine-releasing property.

Effect on smooth (bronchial) muscle

MONGAR and SCHILD (1952) demonstrated contraction of the isolated ileum and uterus of the guinea-pig on administration of 48/80 (0.5 mg/ml). They also found that high concentrations of compound 48/80 (10 mg/ml), released all available histamine from the uterus. However, after the high concentration of 48/80, administration of an antigen still provoked contraction of the uterus.

Compound 48/80 causes obstruction of the lower respiratory tract in the guinea-pig when given as an aerosol (3-5 %) [FEINBERG and STERNBERGER (1955), PAPACOSTA et al. (1959)].

In human experiments, HALPERN (1960) stated that: "patients affected with potential or actual urticaria will respond with a severe generalized urticaria, but not with asthma. In asthmatic patients,

the same dose of the substance produces an asthmatic attack, and only very rarely urticaria". He found asthmatic attacks in allergic individuals following the injection of 0.1 mg/kg 1935-L. However, inhalation of 1935-L by an "asthmatic" does not cause bronchial obstruction (LECOMTE and PETIT, 1960).

Compound 48/80 when given as an aerosol (800 γ/ml for 3 min. with an air flow of 7 lit./min.) does not induce any changes in the VC and/or FEV_1 [personal communication, DE VRIES (1962)] in subjects with affections of the airways.

Effect on blood vessels

PATON (1951) injected 48/80 (10 μg/kg) intravenously in the cat (and dog) and found a delayed hypotension, which has been confirmed by DEWS et al. (1953).

HALPERN (1960) suggested that compound 48/80 does not have a direct effect on the vascular bed, but "that the pressor reponse is a secondary process and related to the release of endogenous histamine. The delayed hypotensive response is one of the characteristic effects of histamine liberators, since it takes time to release endogenous histamine".

Effect on capillary permeability

FELDBERG and MILES (1953) reported increased capillary permeability in the skin of guinea-pigs after intravenous injection of compound 48/80 (1.8-2.2 mg/kg). "There is good evidence that the increased permeability is an effect of released histamine".

MILES and MILES (1952) performed experiments on guinea-pigs concerning vascular reactions to histamine, histamine liberator and leukotaxine in the skin, and concluded: "all three substances increase capillary permeability within 3-5 min. of injection, and their action is mostly finished in 10-15 min. and wholly so in 30 min. Between 15 and 30 min. after the injection, the capillaries not only begin to recover their normal low permeability, but became immune to further doses of the drug . . ."

FELDBERG and MILES (1951) reported that the local effect of compound 48/80 in the guinea-pig's skin, is due to the local release of

histamine and found beside other parts, increased permeability confined to the trachea and bronchi, but not in the lung.

Effect on mucous glandular secretion

Administration of histamine liberators (e.g. 1935-L) causes increase of gastric secretion (HALPERN, 1960). However, no report has been found indicating alterations in nasal mucous glandular secretion following the administration of 48/80, in fact MÉLON and LECOMTE (1958) and MÉLON (1963) reported no change in the nasal secretion of allergic individuals on administration of 1935-L.

Effect on eosinophils

No study has shown yet that 48/80 has any chemotactic effect on eosinophils.

Own clinical experiments

A study aimed at detecting a reaction of the nasal respiratory mucosa to 48/80 was carried out.

Table 53

Comparative effect of histamine and compound 48/80 on the nasal respiratory mucosa by means of topical application in 10 patients with nasal complaints. (4 had also chest complaints).

No.	Histamine reactivity expressed as x of 2^x mg/ml	P_I in cmH_2O	Pharynx-nostril pressure gradient after application of 48/80	Observation					
				Histamine			48/80		
				O	N	S	O	N	S
1	—2	3.5	3.6	++	—	++	—	—	—
2	—1	4.6	3.4	+++	—	++	—	—	—
3	0	5.6	5.6	++	—	+	—	—	—
4	—3	7.0	6.8	+++	+	++	—	—	—
5	1	6.0	6.0	++	—	+	—	—	—
6	—2	4.5	4.5	+++	—	+	—	—	—
7	—2	3.5	3.5	+++	—	—	—	—	—
8	1	4.0	4.5	+++	—	+	—	—	—
9	0	3.8	4.0	+++	—	+	—	—	—
10	—1	3.6	3.6	+++	—	—	—	—	—

O : Swelling of the mucosa
N : Sneezing
S : Hypersecretion
P_I : Initial pressure value
— : No reaction
\+ : Slight
++ : Moderate
+++ : Severe

Ten patients with nasal complaints (obstruction, sneezing and hypersecretion; 4 of these patients had also chest complaints: dyspnoea, cough and sputum) were studied using the methods as described before. The next day after the determination of the histamine reactivity of the nasal mucosa, 1 mg/ml 48/80 was applied for 10 min. to the nasal respiratory mucosa of these patients, under comparable conditions.

The results are summarized in table 53.

Conclusion

Compound 48/80 *had no effect on the nasal respiratory mucosa.* This is in accordance with the results of MÉLON and LECOMTE. (See also chapter VIII, § 3).

§ 6. Adrenaline and isoproterenol (isoprenaline)

1. *Adrenaline*

OLIVER and SCHÄFER in 1895 noted a marked rise in blood pressure following the injection of "extracts of the suprarenal capsules" into animals. The experiments of these investigators initiated extensive investigations by ABEL and CRAWFORD (1897, 1899), VON FÜRTH (1900), TAKAMINE (1901), ALDRICH (1905), etc.. ABEL and CRAWFORD (1899) named this active principle - epinephrine and ALDRICH (1905) called it adrenaline.

BARGER and DALE (1910) introduced the term "sympathomimetic" to describe the type of action of chemically related compounds.

Generally, the responses to adrenaline resemble those produced by stimulation of sympathetic nerves and according to GROLLMAN (1960), less than a millimicrogram per ml. of adrenaline (and noradrenaline) is normally present in the peripheral blood and is largely concentrated in the adrenal medulla. Adrenaline is assumed to be the "emergency hormone" of the sympathetic nervous system.

According to AHLQUIST (1948, 1962) the responses to adrenaline are due to activation of two different types of receptors, namely, α- and β-receptors. In general it can be said that the activation of

α- and β-receptors gives opposite effects: viz. effects of α-receptors are stimulating effects while those of β-receptors are inhibitory effects (PRATESI and GRANA, 1965). According to these authors, the stimulating effect of the α-receptors corresponds to a depolarization (membrane effects) while that of the β-receptors can "be interpreted as linked to repolarization phenomena".

Effect on smooth (bronchial) muscle

It is generally accepted today that adrenaline relaxes the bronchial smooth muscle in man [GADDUM (1953), GOODMAN and GILMAN (1956), YOUNG (1957), LAURENCE and MOULTON (1960), GROLLMAN (1960), BECKMAN (1961), SICE (1962), ARIËNS (1964), PRATESI and GRANA (1965), etc.].

Usually 0.2-0.5 ml of a 1:1000 solution subcutaneously (or a 1 % solution as a spray) is sufficient to produce a bronchodilating effect.

Effect on blood vessels

Interaction of adrenaline with α-receptors, causes vasoconstriction while its effect on β-receptors causes vasodilatation in man (GINSBURG and COBBOLT, 1960). The β-receptors are activated by very low concentrations of adrenaline (vasodilatation) while higher concentrations activate the α-receptors (vasoconstriction) [LAURENCE and MOULTON (1960), GINSBURG and COBBOLD (1960), GOTH (1961), etc.]. However, when the α-receptors are blocked e.g. by 3 mg dibenyline [infusion experiments in man by GINSBURG and COBBOLD (1960) and COBBOLD et al. (1960)], marked dilatation occurs. These authors stated: "after dibenyline, when the action of the α-receptors is inhibited and the only point of attack remaining for adrenaline is on the β-receptor, marked dilatation is observed".

Adrenaline applied to the nasal mucosa (1:1000) causes vasoconstriction [LEGIER (1958), BECKMAN (1961), ARIËNS (1964), etc.].

Effect on capillary permeability

Adrenaline reduces capillary permeability [LAURENCE and MOULTON (1960), ZWEIFACH (1961), etc.].

Effect on mucous glandular secretion

Adrenaline stimulates the excretion of submaxillary and sublingual saliva [GADDUM (1953), BECKMAN (1961), GOTH (1961), SICE (1962), etc.].

In chapter I, it has been stated that section of the parasympathetic nerve fibres (greater superficial petrosal nerve) results in a shrunken nasal mucosa with a decreased secretion (sympathetic effect), however, no study has confirmed that adrenaline decreases the secretion of the nasal glands.

Effect on eosinophils

Eosinopenia has been demonstrated in laboratory animals [e.g. GODLOWSKI (1948), SPEIRS and MEYER (1949), SCHWEIZER (1953)] and in man following the administration of adrenaline [GODLOWSKI (1948), RECANT, FORSHAM and THORN (1948), etc.].

"It has been shown that the subcutaneous injection of the usual therapeutic dose of epinephrine causes a marked decrease in the number of circulating eosinophils" (GOTH, 1961).

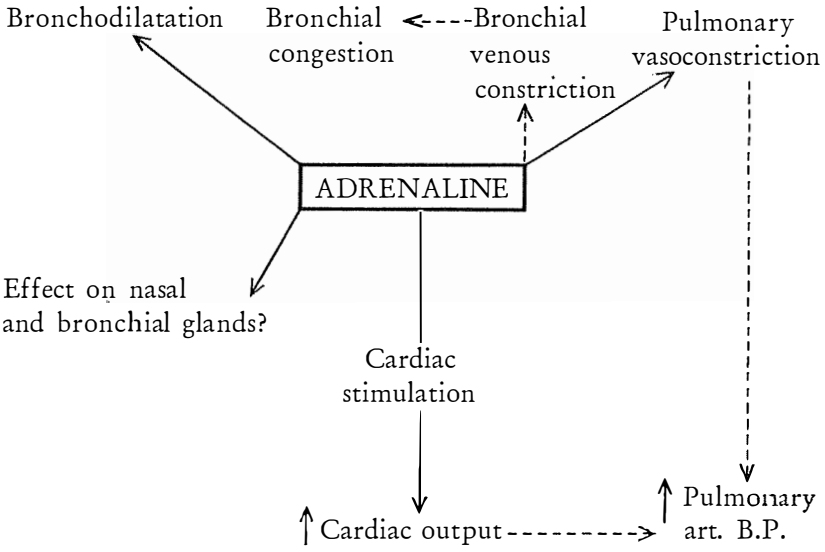


Fig. 17. Actions of Adrenaline
[After AVIADO, 1965 (slightly modified)].

2. *Isoproterenol*

Isoproterenol has first been studied by KONZETT (1940) and has been isolated by LOCKETT (1954) from the adrenals of the monkey, cat and man. The effects of noradrenaline, isoproterenol and adrenaline are classified as α- and β-effects. In this regard, GADDUM (1953) said: "noradrenaline has powerful α-effects (e.g. vasoconstriction in the skin and viscera, contraction of various other plain muscles and inhibition of the intestine) and feeble β-effects (e.g. vasodilatation in muscles, dilatation of bronchi). Isoproterenol has feeble α-effects and powerful β-effects. Adrenaline has intermediate properties".

The bronchodilator action of isoproterenol (1:100) has been found to be more or less 10 times that of adrenaline (1:100) [HAWKINS and SCHILD (1951), GROLLMAN (1960), GOTH (1961)].

BECKMAN (1961) stated that isoproterenol is used practically exclusively as a bronchodilator in asthma. He said: "the chemical structure of isoproterenol has peculiarly specialized it for vasodepressor without vasoconstrictor action . . .".

"Intravenous infusion of isoproterenol in man lowers peripheral vascular resistance . . . It prevents or relieves bronchoconstriction" (INNES and NICKERSON, 1965). These authors also emphasized the fact that isoproterenol is the most active of the sympathomimetic amines acting almost exclusively on β-receptors - that means according to these investigators, its main action is on the heart, smooth muscle of bronchi, skeletal muscle vasculature, and alimentary tract. "Even though isoproterenol is a powerful stimulant of the heart muscle, causing tachycardia and increased cardiac output, it actually produced peripheral vasodilatation and a fall of blood pressure . . ." (GOTH, 1961). BECKMAN (1961) is of the same opinion. Isoproterenol stimulates the myocardium accompanied by an increase in cardiac contractility (contractile force, cardiac work and oxygen consumption) [BERNE (1958), LAURENCE and MOULTON (1960), HASHIMOTO et al. (1960), BECKMAN (1961), WINBURY (1964), etc.].

Effect on smooth (bronchial) muscle

Isoproterenol relaxes the smooth muscles of the bronchi in man,

especially when applied as an aerosol (1:200) [HAWKINS and SCHILD (1951), AVIADO and SCHMIDT (1957), LAURENCE and MOULTON (1960), GROLLMAN (1960), BECKMAN (1961), VAN DER BIJL (1961), GOTH (1961), SICE (1962), SEGAL et al. (1965), etc.].

"All the β-type effects, when considering smooth muscle, are of an inhibitory type, and are shown by the elimination of a pre-existing state of stimulation, they can therefore be interpreted as linked to repolarization phenomena" (PRATESI and GRANA, 1965).

Effect on blood vessels

LEGIER (1958) carried out experiments on the nasal blood vessels of the dog. Isoproterenol (1.6 μg) has been injected first in the vena saphena magna and afterwards in the right common carotid artery (same dose). After the first injection (v. saphena magna) a drop in arterial pressure and nasal vasoconstriction have been observed. After the injection of isoproterenol in the common carotid artery a drop of arterial pressure and "protracted nasal dilatation" have been observed. These injections have been repeated after cutting of the vago-sympathetic nerves: the intravenous injection (1.6 μg) produced dilatation of the nasal blood vessels and a steep fall in arterial pressure; following the injection of 1.6 μg isoproterenol in the common carotid artery, LEGIER found the blood pressure slightly affected accompanied by a marked nasal vasodilatation. He concluded: "isoproterenol has a dilating action on the nasal vascular bed. The hypotension caused by the intravenous injection of isoproterenol elicits a primary reflex nasal vasoconstriction, that may be suppressed by the interruption of the vasoconstrictor fibres of the sympathetic nerve".

Isoproterenol causes vasodilatation in man. "Any amount of isoproterenol always produces vasodilatation" (SICE, 1962).

GINSBURG and COBBOLD (1960) compared the effect of noradrenaline, adrenaline and isoproterenol on forearm blood flow in man by means of intravenous and intra-arterial infusions. Isoproterenol has been applied in a rate of 4 μg/min. intravenously and 1/10 μg/min. intra-arterially for 5 min.. These authors concluded: "... isoprenaline has a direct vasodilator action, the general pattern of response being similar during intravenous or intra-arterial infu-

sions". Similar experiments in man have been reported by COBBOLD, GINSBURY and PATON (1960), who stated: "the fact that dilatation occurred after intravenous and direct intra-arterial infusion, and in healthy and sympathectomized subjects, showed further that it was a direct effect of the drug".

"Isoproterenol is a pure vasodilator whereas epinephrine a dilator for most vessels, but constrictor for the kidney, skin and lung vessels. The bronchomotor effects are accompanied by dilatation of the pulmonary vessels which is desirable (a fall in pulmonary arterial pressure results and the load on the right ventricle is therefore reduced)" [AVIADO, 1965].

ARIËNS (1964) discussed the pharmacology of bronchodilating and bronchoconstricting drugs and said: "the sympathomimetics are a group of drugs frequently used in diseases in which an obstruction of the respiratory tract plays a part. In the case of vasomotor rhinopathy, compounds with a vasoconstrictive action are used as decongestants. This implies the use of compounds with an α-sympathomimetic activity. In the case of bronchial asthma a bronchodilating activity is primarily wanted, which implies the use of β-sympathomimetic compounds . . . Adrenaline, a compound with α-sympathomimetic and β-sympathomimetic actions, is used as a decongestant in rhinopathy and as a bronchospasmolytic in bronchial asthma" (see table 54).

Effect on capillary permeability

It is not known whether isoproterenol has any direct effect on capillary permeability.

Effect on mucous glandular secretion

No report was found concerning the effect of isoproterenol on nasal mucous glandular secretion.

Effect on eosinophils

No evidence exists that isoproterenol has any chemotactic effect on eosinophils.

Table 54

A differentiation in the effects of noradrenaline, adrenaline and isoproterenol.

Organs	Noradrenaline and adrenaline on α-receptors; α-sympathomimetic actions	Adrenaline and isoproterenol on β-receptors; β-sympathomimetic actions
Heart		augmentation myocardial contraction, tachycardia
Muscular vessels	slight decrease in blood flow, vasoconstriction	strong increase in blood flow, vasodilation
Brain vessels (human)	decrease in blood flow, vasoconstriction	increase in blood flow, vasodilation
Vessels splanchnic area	strong decrease in blood flow, vasoconstriction	slight increase in blood flow
Splenic capsule	contraction	
Renal vessels	strong decrease in blood flow	
Cutaneous vessels	strong decrease in blood flow, vasoconstriction	slight increase in blood flow
Vessels nasal mucosa	vasoconstriction	vasodilation
Pilomotor muscle	contraction (raising of hairs)	
Bronchial tree		relaxation bronchial muscle
Intestine	relaxation intestinal smooth muscle	relaxation intestinal smooth muscle
Ureter	contraction	
Vas deferens	contraction	
Uterus	excitation, uterine contractions (depending on condition of uterus; promoted by oestrogens)	inhibition uterine contractions
Dilator muscle iris	contraction (mydriasis)	
Membrana nictitans	contraction	
Carbohydrate metabolism	increase blood-sugar level (glycogenolysis liver)	increase blood-lactic acid level (glycogenolysis muscle)
Fat metabolism	mobilization of fat (shift from depots to liver)	

(After Ariens, 1964).

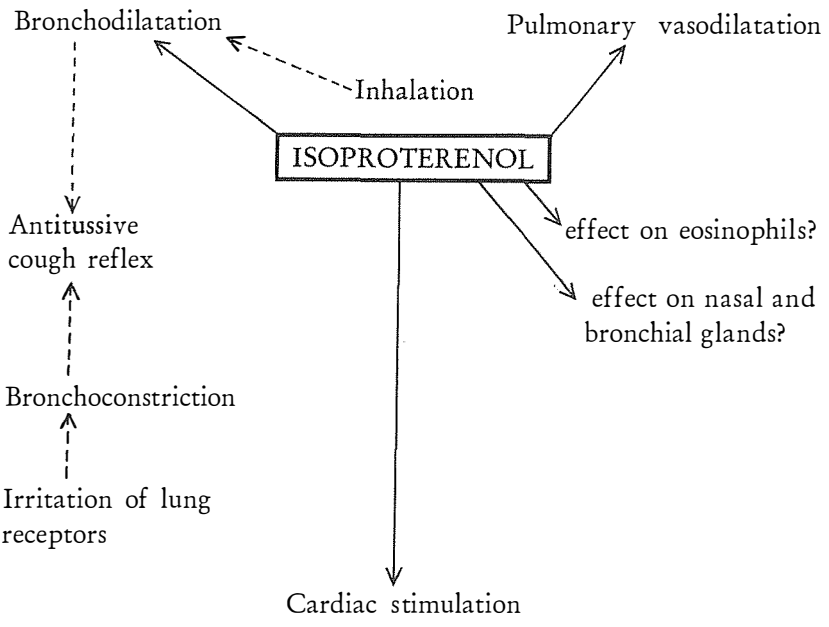


Fig. 18. Actions of Isoproterenol
[After AVIADO, 1965 (slightly modified)].

Own clinical experiments

The effect of isoproterenol sulphate and adrenaline on the nasal respiratory mucosa was studied in 20 patients with nasal complaints, and known nasal histamine reactivity.

Using the same method as described in the preceding experiments, isoproterenol sulphate (1:100) was applied to the nasal respiratory mucosa for 1 min. in 8 patients. In 12 patients, isoproterenol sulphate was applied (1:100) after partial nasal obstruction induced by histamine application. After each application of isoproterenol sulphate, adrenaline (1:1000) was applied to the nasal respiratory mucosa for 45 sec..

The results are shown in table 55.

Conclusion

Isoproterenol caused a *congestion of the nasal mucosa,* while adrenaline induced a *marked decongestion.*

Table 55

Comparative effect of isoproterenol sulphate, histamine and adrenaline on the nasal respiratory mucosa by means of topical application in 20 patients with nasal complaints.

		Pharynx-nostril pressure gradient in cmH_2O after application of:			
		Isoproterenol*		Isoproterenol*	
No.	P_I	sulphate (1 : 100)	Histamine	sulphate (1 : 100)	Adrenaline** (1 : 1000)
1	5.6	7.0	—	—	3.5
2	6.6	8.6	—	—	7.0
3	3.5	5.8	—	—	4.5
4	2.8	5.0	—	—	3.6
5	7.0	9.5	—	—	5.3
6	4.5	8.6	—	—	3.5
7	3.6	6.5	—	—	4.5
8	5.6	7.5	—	—	6.0
9	3.4	—	22.5	25.6	7.0
10	5.6	—	33.2	38.0	5.6
11	3.4	—	21.0	24.0	4.5
12	7.5	—	28.5	31.6	8.0
13	8.6	—	33.6	35.4	3.5
14	5.6	—	25.3	28.6	4.6
15	7.0	—	38.6	42.5	6.5
16	6.5	—	27.8	30.0	7.5
17	5.6	—	21.0	22.5	6.0
18	4.5	—	36.5	40.0	8.0
19	3.6	—	27.5	31.5	5.6
20	5.6	—	29.5	34.0	7.5

* 0.018 Mol/lit.
** 0.005 Mol/lit.
P_I: Initial pharynx-nostril pressure gradient.

Chapter VI

EXPERIMENTAL INVESTIGATION ON THE EFFECT OF ALLERGENS ON THE NASAL RESPIRATORY MUCOSA AND BRONCHIAL TREE

§ 1. Introduction

In chapter IV the relation between the histamine reactivity of the nasal respiratory mucosa and nasal complaints has been discussed.

The nasal complaints viz. obstruction, sneezing and hypersecretion fit in with the clinical picture as seen in "vasomotor rhinitis" and pollinosis.

In chapter II, a number of pathogenic factors of rhinopathy, among which the allergic factor, has been discussed.

The allergic reaction is characterized by an antigen (allergen) - antibody union, by which histamine (and histamine-like substances) is released. From the clinical point of view allergens can be classified by their mode of entrance into the human body.

1. *Inhalants* — allergic reactions induced by inhalant allergens, are predominantly localized in the nasal mucosa and bronchial tree. Examples of inhalants: pollen, moulds, house dust, epidermals, etc..
2. *Ingestants* — e.g. foods and drugs.
3. *Injectants* — toxins, serum, vaccines, non-specific proteins, etc..
4. *Contactants* — chemical compounds, heavy metals, dyes, etc..

Ingestants, injectants and contactants probably play a minor rôle in the reactions of the nasal mucosa or bronchial tree.

On primary contact of the nasal mucosa (bronchial tree) with a specific allergen, in certain predisposed individuals, formation of a special kind of specific antibodies occurs in the antibody-forming immature plasma cells, with the property of fixation on the surface of cells. Re-exposure to the specific allergen leads to a union of this allergen with the cell-fixed specific antibody with liberation of

histamine (and histamine-like substances), the ultimate effect of which can be observed as sneezing, hypersecretion and nasal obstruction (bronchial obstruction).

The response of the nasal mucosa and bronchial tree to allergens can be studied directly by means of provocative tests.

The nasal provocative test

The earliest nasal provocative test recorded in medical annals, was made in December 1835 by KIRKMAN, who sniffed sweet vernal grass and so developed symptoms of "hay fever". Thirty eight years later, BLACKLEY was the first who carried out systematic provocative tests:

1. nasal test
2. buccal instillation test
3. conjunctival test
4. bronchial inhalation test
5. inoculation test

In 1903, DUNBAR — although his chief reliance was based on the conjunctival test — applied also the nasal provocative test to ascertain which were the offending pollens and what was the degree of sensitivity. Later on nasal provocative tests were studied by various investigators as, DUKE (1924, 1925), VAN DER VEER et al. (1927), EFRON and PENFOUND (1930), COCA, WALZER and THOMMEN (1931), PEHU and WORINGER (1933), URBACH (1933), RUDOLPH and COHEN (1934), DEAN, LINTON and LINTON (1935), BLUMSTEIN (1937, 1945), ROWE (1937), TUFT (1937), CHOBOT et al. (1940), HARRIS (1941), VAN DISHOECK (1942), TUFT and BLUMSTEIN (1950), FEINBERG, STIER and GRATER (1952), HALPERN, HOLMAN and WHITTAKER (1961), TUFT et al. (1962).

These tests were carried out in different forms: by sniffing the allergen held close to the patient's nose, or spraying it into the nostril; by installing a cotton wad moistened with the allergen into a nasal chamber; by application of the allergen to a scratch in the mucosa or by injecting an allergen solution into the epithelium.

The bronchial provocative test

In 1931, PEIPERS introduced the bronchial inhalation test to de-

termine pulmonary sensitivity to house dust. Following this, many reports concerning the bronchial inhalation test have been published e.g. STEVENS (1934), HARRIS (1941), LOWELL and SCHILLER (1948), JUHLIN-DANNFELT (1950), TUFT and BLUMSTEIN (1950), SCHLEINZER (1951), HERXHEIMER (1951), COLLDAHL and LUNDIN (1952), COLLDAHL (1952), TEN CATE (1954), TIFFENEAU (1955, 1960), CITRON, FRANKLIN and SINCLAIR (1958), DUCHAINE and SPAPEN (1959), BILLIET et al. (1959), MICHGELSEN (1959), FASTMAN and GLAZER (1963), BRUCE (1963), ITKIN et al. (1963), KREUKNIET and YOUNG (1964), PAULSEN (1965), GEUBELLE et al. (1965), BEUMER (1965), ADO (1965), KIM (1965).

The evaluation of either the nasal or bronchial provocative test, however, renders some difficulties. It was demonstrated that histamine is released by the antigen-antibody reaction [DRAGSTEDT and GEBAUER-FUELNEGG (1932), CODE (1939), KATZ and COHEN (1941), SCHILD et al. (1951), etc.]. This supports the postulation that the response induced by a provocative test with an allergen will be greater in an effector organ (e.g. nasal mucosa or bronchial tree) — which has also an increased susceptibility to exogenous histamine — than, when an increased histamine susceptibility does not exist. The experiments by DE VRIES et al. with respect to the bronchial tree, support this statement.

Even if substances other than histamine are released, it is conceivable that this hypothesis holds true, provided that the histamine reactivity is taken as a graduator for other non-antigenic stimuli.

Therefore, in assessing the bronchial reactivity to allergens TIFFENEAU takes into account the bronchial histamine-acetylcholine reactivity (l'hyperexcitabilité bronchomotrice). The relationship, however, between the aspecific component (histamine reactivity) and the degree of sensitization for allergens is unknown in the case of the nasal allergen provocative test.

Neither the intensity of the reaction, nor the dosis applied, is a direct measurement for this allergic sensitization. Therefore, the relationship between the aspecific nasal reactivity and the state of specific sensitization of the nasal mucosa was the subject of this study.

Provocatieve experiments were done with allergens (grass pollen, house dust, moulds and epidermals) and the reactivity of the nasal mucosa to histamine was also assessed.

An attempt was made to analyse the following questions.

A. whether there is a relation between the result of the nasal provocative test with allergens and the clinical condition (e.g. presence or absence of a history of manifest pollinosis; presence or absence of a history of nasal complaints).
B. whether a relationship exists between the reaction of the nasal mucosa to allergens and to histamine.
C. whether a relationship exists between the reaction of the nasal mucosa and bronchial tree to allergens.

§ 2. Methods and materials

The patients for this investigation were selected from those attending the Pulmonary Division of the Department of Medicine and the Department of Oto-Rhino-Laryngology, State University Hospital, Groningen.

Selection of patients

The test subjects for the pollen provocative tests were divided according to:

— the presence or absence of manifest pollinosis defined as the seasonal (May till half August) recurrence of characteristic nasal symptoms: excessive sneezing, increased watery nasal discharge and nasal obstruction;
— the presence of a positive intracutaneous test* to grass pollen;
— age: under and over 40 years.

The test subjects for the provocative tests with house dust, moulds and epidermals were divided according to:

— the presence or absence of nasal complaints** (obstruction, hypersecretion and sneezing attacks);
— the presence of a positive intracutaneous* test to the allergen to be tested;
— age: under and over 40 years.

---

* For the technique and interpretation of the intracutaneous test, see Appendix IV.

** The presence or absence of the nasal complaints was recorded according to the questionnaire given in Appendix I.

Methods of investigation

The following determinations were performed:

a. the histamine reactivity of the nasal mucosa;
b. the response of the nasal respiratory mucosa to grass pollen, house dust, moulds and epidermals;
c. the histamine reactivity of the bronchial tree;
d. the response of the bronchial tree to grass pollen, house dust, moulds and epidermals.

ad a. The method for the assessment of the nasal histamine reactivity is described in chapter III.

ad b. *Assessment of the response of the nasal mucosa to the different allergens*

The same method* as described in chapter III for the assessment of the nasal histamine reactivity was used for the assessment of the nasal response to pollen, house dust, moulds and epidermals.

I. *Pollen*

Different concentrations of pollen extracts were applied, viz. 0.1, 10, 100, 1000, 10.000 Noon Units. The pollen extracts were administered by means of a cotton wad on a probe, placed in a nasal chamber during a time varying from 1-12 min.. After a positive reaction, adrenaline (1 : 1000) was applied to the mucosa for 30-45 sec., after which the reaction subsided.

The assessment of the nasal histamine reactivity and the response to pollen was done on different days under comparable conditions. The same holds true for the bronchial tree. No pollen provocative test was carried out during the pollen season.

II. *House dust*

House dust extract (5 mg/ml) was applied by means of a cotton wad on a probe, placed in a nasal chamber until a reaction of the mucosa occurred; this time varied from 3-12 min. The further procedure was the same as for pollen.

* Coca solution was applied for a placebo reading in this investigation instead of distilled water, since the allergens are dissolved in this solution.

III. *Moulds*

By using the same method as in the case of house dust provocation, 2.5 mg/ml moulds extract was applied. In all the subjects tested a combined standard mould extract (see Appendix V) was used. In 2 patients a negative reaction was obtained following the application of the combined extract, while after the application of moulds "A" extract a positive reaction occurred in these persons.

IV. *Epidermals* (see Appendix V)

A combined extract of epidermals in concentrations of 2.5 mg/ml was applied to the nasal mucosa in the same way as described for the other allergens. Here too, 2 subjects did not react to the combined extract, but did to an extract of cat hair.

ad c. The method for the assessment of the bronchial histamine reactivity is described in chapter III, § 6.

ad d. *Assessment of the reaction of the bronchial tree to pollen, house dust, moulds and epidermals*

All the solutions were nebulized with an air flow of 8 lit./min..

Pollen

Aerosols with mounting concentrations of pollen extracts were inhaled. The concentrations of the pollen extracts used, were: 10, 100, 1000, 10.000 Noon Units.

After the determination of the VC and FEV_1 a control solution (Coca) was inhaled for 10 min., after which the VC and FEV_1 were measured again. These measurements were repeated after 1, 3, 6, 9 and 12 min., and were regarded as the basis values necessary for the pollen challenge test. Hereafter, the pollen extract (10 Noon Units) was inhaled for 1 min., after which the VC and FEV_1 were measured. When a decrease in the VC and/or FEV_1 of not more than 10 % was found, the pollen extract was inhaled again for 3 min., after which the VC and FEV_1 were measured. If again no descrease in the VC and/or FEV_1 (< 10 %) was noted, the inhalation of the pollen extract was prolonged for the next 3 min., and then a futher measurement of VC and FEV_1 were made. If still, no decrease occurred, the inhalation was continued and controlled in the same way, until

the patient had inhaled the pollen aerosol for a maximum of 10 min. When a reaction occurred before the 10 min., the inhalation was discontinued. If, however, a decrease in the VC and/or FEV_1 (not more than 10 %) occurred, the same procedure was done for the next pollen concentration, viz. 100 Noon Units. If no reaction occurred, the same procedure was applied for the inhalation of 1000 and 10.000 Noon Units respectively.

When no change of 10 % or more in the VC and/or FEV_1 was found following the inhalation of 10.000 Noon Units for 10 min., the inhalation provocation test was interpreted as negative.

If, for instance, a positive reaction occurred during the course of the inhalation of 100 Noon Units of the first 3 min., the challenge test was then discontinued and the VC and FEV_1 were measured after 1, 3 and 6 min.. If after that, no positive reaction was measured, the provocation was continued. However, if after the 6 min. the reaction measured was still positive, isoproterenol (1 : 100) was inhaled by the patient for 30 sec., after which the reaction subsided.

House dust, moulds and epidermals

The same method as described for the inhalation challenge test for pollen was applied for the bronchial provocation of the other allergens by using the following concentrations: house dust 5 mg/ml; moulds 2.5 mg/ml; and epidermals 2.5 mg/ml.

§ 3. Results of the provocative tests

I. *Pollen*

A. Relation between the result of the nasal pollen provocative test and the clinical condition (presence or absence of *manifest* pollinosis).

The data of the subjects tested, according to the conditions stated, are represented in table 56.

Conclusion

1. As can be seen in table 56 no patient with complaints of manifest pollinosis was encountered with a negative intracutaneous test to pollen.

Table 56

Representation of the results of the investigation on the reaction of the nasal respiratory mucosa to pollen in 134 individuals. The results are arranged according to

— the presence or absence of manifest pollinosis,
— the presence or absence of chronic non-specific lung disease (CNSLD),
— the presence or absence of a positive intracutaneous test,
— age: under and over 40 years,
— a positive or negative provocative test.

			CNSLD positive				CNSLD negative			
			Reaction of the nasal mucosa				Reaction of the nasal mucosa			
			Positive			Negative	Positive			Negative
	Pollen concentration in Noon units		≦ 100	1000	10.000	> 10.000	≦ 100	1000	10.000	> 10.000
	Intracutaneous test	Age group								
Manifest pollinosis	+	≦ 40	8	10	7	0	2	4	3	0
		> 40	0	3	7	0	0	0	0	0
	—	≦ 40	0	0	0	0	0	0	0	0
		> 40	0	0	0	0	0	0	0	0
No manifest pollinosis	+	≦ 40	0	0	0	12	0	0	0	0
		> 40	0	0	0	11(+ 1)*	0	0	0	0
	—	≦ 40	0	0	0	14(+20)*	0	0	0	9(+ 5)
		> 40	0	0	0	11(+ 2)*	0	0	0	3(+ 2)

( )* Number of subjects, in whom no bronchial pollen provocative tests were done.

2. Positive nasal provocative tests were found only in those subjects with the characteristic complaints of manifest pollinosis (see chapter II, § 1). Persons without these characteristic complaints did not respond to pollen provocation. This finding points to a *specific character* of the nasal provocative test.
3. Generally, in the group of subjects with complaints of manifest pollinosis, and with CNSLD, more individuals were found under 40 years of age, in which the nasal provocative test was positive following the provocation by the lower pollen concentrations, in comparison with those over 40 years of age. However, as manifest pollinosis occurs less frequently in subjects over 40 years of age (see chapter II, § 1), the number of persons represented here in this category is small.

B. The relation between the nasal histamine reactivity and the reaction of the nasal mucosa to pollen.

The results are arranged in table 57.

Table 57

The results of the investigation concerning the relation between the nasal histamine reactivity and the nasal reaction to pollen in the two age groups. The different subgroups are arranged according to the degree of the nasal histamine reactivity, e.g.

subgroup 1: — manifest pollinosis positive (+)
— positive intracutaneous test to grass pollen (+)
— positive CNSLD criteria (+)
— positive nasal provocative test (+)

subgroup 4: only positive criteria for CNSLD; all the other characteristics are negative (—).

	Under 40 years of age Subgroup					Over 40 years of age Subgroup				
	1	2	3	4	5	1	2	3	4	5
Manifest pollinosis	+	+	—	—	—	+	+	—	—	—
Intracutaneous test to grass pollen	+	+	+	—	—	+	+	+	—	—
CNSLD	+	—	+	+	—	+	—	+	+	—
Nasal provocative test	+	+	—	—	—	+	+	—	—	—
N_{HR}										
6	0	0	0	0	1	0	0	0	0	0
5	0	0	1	0	2	0	0	1	0	1
4	0	0	0	2	3	0	0	0	0	1
3	0	0	0	2	1	0	0	0	2	1
2	0	0	0	1	1	0	0	0	2	0
1	0	0	0	0	1	0	0	1	3	1
0	2	0	1	5	1	1	0	2	2	0
—1	5	3	4	8	4	5	0	2	1	0
—2	2	3	1	10	0	1	0	3	3	0
—3	10	2	4	5	0	3	0	2	0	0
—4	6	1	1	1	0	0	0	1	0	0
Mean	—2.5	—2.1	—1.4	—0.9	+1.4	—1.6		—1.4	+0.9	+3.8

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

From table 57 a difference seems to exist with respect to the degree of the nasal histamine reactivity of the corresponding subgroups in the two age groups. Therefore, the above table has been contracted to analyse this possible "age effect" on the degree of the histamine reactivity in the subgroups (see table 58). However, no significant "age effect" was found ($\chi^2_6 = 9.57$).

Table 58

The comparison of the degree of the nasal histamine reactivity of the corresponding subgroups in the two age groups (for explanation of the subgroups, see table 57).

	≦ 40 years of age Subgroup				> 40 years of age Subgroup			
	1 & 2	3	4	5	1	3	4	5
Manifest pollinosis	+	—	—	—	+	—	—	—
Intracutaneous test to grass pollen	+	+	—	—	+	+	—	—
CNSLD	+	+	+	—	+	+	+	—
Nasal provocative test	+	—	—	—	+	—	—	—
N_{HR}								
≧ —1	7	6	18	14	6	6	10	5
≦ —2	18	6	16	0	4	6	3	0

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

The mean value for the degree of the nasal histamine reactivity in subgroup 5 over 40 years of age (no manifest pollinosis, negative intracutaneous test to pollen, negative criteria for CNSLD and negative nasal provocative test) resembles very closely the "normal" value which has been inferred from the epidemiological study (see chapter IV).

No significant difference between *subgroup 1* (manifest pollinosis, positive pollen intracutaneous test, positive criteria for CNSLD and positive nasal provocative test) and *supgroup 2* (manifest pollinosis, positive pollen intracutaneous test, criteria for CNSLD negative and positive nasal provocative test) under 40 years of age was found, concerning the degree of the nasal histamine reactivity.

Since no clear "age effect" was found, the two age groups were arranged in one group (see the next table).

From table 59 a striking difference appears to exist between the degree of the nasal histamine reactivity of the subjects with manifest pollinosis and those without this condition: the subgroup characterized by manifest pollinosis, positive intracutaneous test to pollen,

Table 59

A comparison of the degree of the nasal histamine reactivity in subjects with and without manifest pollinosis.

	Subgroup			
	1 & 2	3	4	5
Manifest pollinosis	+	—	—	—
Intracutaneous test to grass pollen	+	+	—	—
CNSLD	+ & —	+	+	—
Nasal provocative test	+	—	—	—
N_{HR}				
$\geqq$ —1	16	12	28	19
$\leqq$ —2	28	12	19	0

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

CNSLD positive or negative and nasal provocative test positive, namely subgroup 1 & 2 shows an increased histamine reactivity of the nasal mucosa in comparison with subgroup 4* ($p = 0.05$) and subgroup 5* ($p = 0.001$), however, not with subgroup 3* — and this finding, probably indicates that beside the occurrence of manifest pollinosis — the sensitization without symptoms may also influence the degree of the nasal histamine reactivity. However, this could not statistically be confirmed. Furthermore, when comparing subgroup 3 and 4 with respect to the degree of the nasal histamine reactivity, there appears to be no significant difference. Yet, a significant difference exists between subgroup 4 and 5. From this finding, however, it cannot be concluded that CNSLD has a relevant influence on the nasal histamine reactivity, since all those subjects with positive criteria for CNSLD had nasal complaints.

Since a significant difference exists between subjects with and without manifest pollinosis in respect to the degree of the nasal histamine reactivity, a further analysis was done to see if a correlation exists between the nasal threshold values for pollen and the nasal histamine reactivity (see table 60).

Table 60

The relation between the threshold values of the nasal mucosa to pollen and the degree of the histamine reactivity.

Nasal histamine reactivity	Pollen concentration in Noon units	
expressed as x of 2^x mg/ml	$\leqq$ 1000	10.000
$\geqq$ —2	5	10
$\leqq$ —3	19	0

Subgroup 3: no manifest pollinosis, positive skin tests to pollen, positive criteria for CNSLD and negative nasal provocative tests.
Subgroup 4: subjects only with positive criteria for CNSLD without the other characteristics.
Subgroup 5: all characteristics negative.

Table 60 clearly demonstrates that the lowest pollen concentrations causing positive nasal reactions, are strongly correlated with the degree of the nasal histamine reactivity ($p = 0.001$).

C. The relation between the reaction of the nasal respiratory mucosa and the bronchial tree to pollen.

The results of the nasal and bronchial grass pollen provocative tests, performed in a number of subjects in the subgroups of the two age groups are represented in table 61.

Table 61

The results of the nasal and bronchial pollen provocative tests in the various subgroups under and over 40 years of age. The subgroups are characterized by the presence or absence of:

— manifest pollinosis
— positive intracutaneous tests to pollen
— CNSLD

		≦ 40 years of age Subgroup					> 40 years of age Subgroup			
		1	2	3	4	5	1	3	4	5
Manifest pollinosis		+	+	—	—	—	+	—	—	—
Intracutaneous test to pollen		+	+	+	—	—	+	+	—	—
CNSLD		+	—	+	+	—	+	+	+	—
		AL_B	AL_B	AL_B	AL_B	AL_B	AL_B	AL_B	AL_B	AL_B
		+ —	+ —	+ —	+ —	+ —	+ —	+ —	+ —	+ —
AL_N	+	25 0	9 0	0 0	0 0	0 0	8 2	0 0	0 0	0 0
	—	0 0	0 0	0 12	0 14	0 9	0 0	9 2	0 11	0 3

AL_N: Nasal provocative test, positive (+) or negative (—).
AL_B: Bronchial provocative test, positive (+) or negative (—).

Conclusion

1. In all the subjects with manifest pollinosis, a positive nasal as well as a positive bronchial provocative pollen test was obtained, except in 2 subjects over 40 years of age who did not respond to the inhalation of pollen.
2. In all the subjects in the subgroups under 40 years of age without manifest pollinosis, no positive nasal or bronchial provocative test was encountered. However, in subgroup 3 (subjects over 40 years of age without manifest pollinosis anymore, with positive

intracutaneous tests to pollen and positive criteria for CNSLD), 9 patients were found who reacted with bronchial obstruction to the inhalation of pollen extracts, while the nasal pollen provocative tests were negative. In these patients an increased aspecific excitability of the bronchial tree probably played a part in the event of bronchial narrowing.

II. *House dust*

A. The relation between the result of the nasal provocative test and the clinical condition (presence or absence of nasal complaints).

A survey of the nasal provocative tests with house dust, is represented in table 62.

Table 62

A representation of the positive and negative nasal provocative tests with house dust, arranged according to:
— the presence or absence of nasal complaints*
— the presence or absence of positive intracutaneous tests to house dust
— age: under and over 40 years
— the presence or absence of CNSLD

* Nasal complaints	Intracutaneous test to house dust (i.c.)	Age groups	CNSLD positive Nasal provocative test		CNSLD negative Nasal provocative test	
			Positive	Negative	Positive	Negative
Positive	i.c. +	≦ 40	38	31	0	0
		> 40	7	16	0	0
	i.c. —	≦ 40	0	12	0	5
		> 40	0	8	0	1
Negative	i.c. +	≦ 40	0	1	0	5
		> 40	0	2	0	0
	i.c. —	≦ 40	0	0	0	12
		> 40	0	0	0	5

* Nasal obstruction, nasal hypersecretion and sneezing attacks, recorded according to the questionnaire given in Appendix I (the same holds true for moulds and epidermals).

Conclusion

1. A positive nasal provocative test with house dust, was found to occur only in subjects with nasal complaints.

2. A negative nasal provocative test was found to occur, not only in subjects without nasal complaints, but also in those with nasal complaints.
3. A positive nasal provocative test with house dust was not found in subjects with negative intracutaneous tests to house dust.
4. There seems to be an "age effect" with respect to the positive nasal provocative tests obtained in those patients with nasal complaints, CNSLD and positive intracutaneous tests to house dust — although statistically, not significant ($\chi^2_1 = 3.34$).
5. Subjects characterized by: positive nasal complaints and intracutaneous tests to house dust without CNSLD, are not represented here, probably as a consequence of selection.

B. The relation between the nasal histamine reactivity and the reaction of the nasal mucosa to house dust.

In table 63 the data are given with respect to the relation between

Table 63

The results of the investigation concerning the relation between the nasal histamine reactivity and the nasal reaction to house dust in the two age groups. The different subgroups are arranged according to the degree of the nasal histamine reactivity.

The subgroups are characterized as follows:
eg. subgroup 1:
— positive nasal complaints (+)
— positive intracutaneous test to house dust (+)
— positive criteria for CNSLD (+)
— positive nasal provocative test (+)

	Under 40 years of age Subgroup							Over 40 years of age Subgroup					
	1	2	3	4	5	6	7	1	2	4	5	6	7
Nasal complaints	+	+	—	+	—	+	—	+	+	+	—	+	—
I.c. test to house dust	+	+	+	—	+	—	—	+	+	—	+	—	—
CNSLD	+	+	—	+	+	—	—	+	+	+	+	—	—
Nasal provocative test	+	—	—	—	—	—	—	+	—	—	—	—	—
N_{HR}													
6	0	0	1	0	0	0	3	0	0	1	0	0	2
5	0	0	2	0	0	1	4	0	0	0	0	0	2
4	0	0	1	1	0	0	2	0	0	1	0	0	2
3	0	1	0	0	0	3	2	0	2	1	1	0	0
2	0	3	0	2	0	0	1	0	2	0	0	0	0
1	0	2	1	0	0	0	0	0	3	0	0	1	0
0	1	6	0	1	1	1	0	0	3	1	1	0	0
—1	5	11	0	4	0	0	0	6	2	1	0	0	0
—2	9	4	0	3	0	0	0	0	2	1	0	0	0
—3	16	4	0	1	0	0	0	1	1	2	0	0	0
—4	7	0	0	0	0	0	0	0	1	0	0	0	0
Mean	—2.7	—0.6	+4.2	—0.4		+2.8	+4.5	—1.2	0	+0.5	+1.5	+1.0	+5.2

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

the degree of nasal histamine reactivity and the reaction of the nasal mucosa to house dust in different subgroups.

When looking to the mean values, there seems to be a difference in the subgroups of the two age groups concerning the degree of the nasal histamine reactivity. For the statistical analysis, table 63 has been contracted into table 64.

Table 64

The comparison of the degree of the nasal histamine reactivity in the subgroups of the two age groups (for explanation of the subgroups, see table 63).

	≦ 40 years of age Subgroup							> 40 years of age Subgroup					
	1	2	3	4	5	6	7	1	2	4	5	6	7
Nasal complaints	+	+	—	+	—	+	—	+	+	+	—	+	—
I.c. test to house dust	+	+	+	—	+	—	—	+	+	—	+	—	—
CNSLD	+	+	—	+	+	—	—	+	+	+	+	—	—
Nasal provocative test	+	—	—	—	—	—	—	+	—	—	—	—	—
N_{HR}													
≧ —1	6	23	5	8	1	5	12	6	12	5	2	1	5
≦ —2	32	8	0	4	0	0	0	1	4	3	0	0	0

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

Conclusion

1. A marked difference was found between subgroup 1 and 2 under 40 years of age with respect to the degree of the nasal histamine reactivity, namely, *subgroup 1* (characterized by positive nasal complaints, positive intracutaneous tests to house dust, positive criteria for CNSLD and positive nasal provocative tests to house dust) showed an increased nasal histamine reactivity in comparison with *subgroup 2* (characterized by positive nasal complaints, positive intracutaneous tests to house dust and with CNSLD, but with negative nasal provocative tests to house dust). This striking difference of the nasal histamine reactivity between the first two subgroups under 40 years of age - was not found in the corresponding subgroups over 40 years of age.
2. An "age effect" on the degree of the nasal histamine reactivity was found in subgroup 1, but not in the other subgroups.
3. Comparison of subgroup 4 and 6 in the two age groups, suggests an increased reactivity of the nasal mucosa to histamine in those subjects with CNSLD; however, no conclusion can be made, since the numbers are too small.

4. No significant influence was found in the other comparable subgroups (subgroup 3 versus 7 under 40 years of age, and 2 versus 4 in the two age groups) with respect to the presence or absence of a positive intracutaneous test (as indicator of sensitization) to house dust.
5. The relation between the nasal histamine reactivity and the reaction of the nasal mucosa to house dust, is generally the same as that of pollen provocation.

C. The relation between the reaction of the nasal respiratory mucosa and the bronchial tree to house dust.

A survey of the nasal and bronchial provocative tests accomplished in a number of subjects arranged in subgroups, is represented in table 65.

Table 65

The results of the nasal and bronchial provocative tests with house dust in the various subgroups under and over 40 years of age. The subgroups are characterized by the presence (+) or absence (—) of:
— nasal complaints
— positive intracutaneous tests to house dust
— CNSLD

	≦ years of age Subgroup						> years of age Subgroup				
	1	2	3	4	5	6	1	2	4	5	6
Nasal complaints	+	+	—	+	—	—	+	+	+	—	—
Intracutaneous test to house dust	+	—	+	—	+	—	+	—	—	+	—
CNSLD	+	+	—	—	+	—	+	+	—	+	—

		AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —
AL_N	+	27	11	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0	0
	—	6	23	0	12	0	5	0	5	0	1	0	5	7	9	0	3	0	1	0	2	0	5

AL_N: Nasal provocative test, positive (+) or negative (—)
AL_B: Bronchial provocative test, positive (+) or negative (—)

Conclusion

1. A positive nasal provocative test was found to occur only in subjects with nasal complaints and with positive intracutaneous tests to house dust.

2. A positive bronchial provocative test was found to occur only in those subjects with positive intracutaneous tests to house dust and with CNSLD (subgroup 1).
3. A strong correlation was found between the bronchial tree and nasal mucosa with respect to the positive and negative bronchial and nasal provocative tests in subgroup 1 under 40 years of age.
4. In the group of patients under 40 years of age, significant more positive nasal provocative tests were found than in the group over 40 years of age ($p = 0.05$) while the relation between the positive and negative bronchial provocative tests with house dust in the two age groups, remained fairly constant (see table 66).

Table 66

Comparison between the positive (+) and negative (—) bronchial provocative tests with house dust in the two age groups.

		≤ 40 years of age	> 40 years of age
Bronchial provocative test	+	33	11
	—	34	12

III. *Moulds*

A. The relation between the result of the nasal provocative test and the clinical condition (presence or absence of nasal complaints).

A survey of the results of the nasal provocative tests with moulds, is given in table 67.

Conclusion

1. A positive nasal provocative test with moulds, was found to occur only in subjects with nasal complaints, positive intracutaneous tests to moulds and with CNSLD.
2. In the group of patients with nasal complaints, positive intracutaneous tests to moulds and with CNSLD, an "age effect" was found with respect to the positive nasal provocative tests; significant more positive nasal provocative tests were found to occur in the group under 40 years of age ($p = 0.007$).

B. The relation between the nasal histamine reactivity and the reaction of the nasal mucosa to moulds.

Table 67

A representation of the positive and negative nasal provocative tests with moulds, arranged according to:

— the presence or absence of nasal complaints
— the presence or absence of positive intracutaneous tests to moulds
— age: under and over 40 years
— the presence or absence of CNSLD

Nasal complaints	Intracutaneous test to moulds (i.c.)	Age groups	CNSLD positive Nasal provocative test		CNSLD negative Nasal provocative test	
			Positive	Negative	Positive	Negative
Positive	+	≦ 40	15	18	0	3
		> 40	2	21	0	0
	—	≦ 40	0	18	0	0
		> 40	0	12	0	0
Negative	+	≦ 40	0	0	0	0
		> 40	0	0	0	0
	—	≦ 40	0	0	0	6
		> 40	0	0	0	4

Table 68

The results of the investigation regarding the relation between the nasal histamine reactivity and the reaction of the nasal mucosa to moulds in the two age groups. The subgroups are arranged according to the degree of the nasal histamine reactivity. For explanation of the characteristics of the subgroups, see table 63.

	Under 40 years of age Subgroup					Over 40 years of age Subgroup			
	1	2	3	4	5	1	2	4	5
Nasal complaints	+	+	+	+	—	+	+	+	—
Intracutaneous test to moulds	+	+	+	—	—	+	+	—	—
CNSLD	+	+	—	+	—	+	+	+	—
Nasal provocative test	+	—	—	—	—	+	—	—	—
N_{HR}									
6	0	0	0	0	2	0	0	0	1
5	0	0	0	0	2	0	0	0	2
4	0	1	0	1	1	0	0	0	1
3	0	1	1	0	1	0	2	2	0
2	0	1	1	1	0	0	2	0	0
1	0	2	0	1	0	0	5	1	0
0	1	0	0	2	0	0	4	3	0
—1	4	8	1	8	0	0	3	3	0
—2	6	3	0	4	0	2	5	2	0
—3	3	1	0	1	0	0	0	1	0
—4	1	1	0	0	0	0	0	0	0
Mean	—1.9	—0.6	+1.3	—0.7	+4.8	—2.0	+1.0	—0.3	+5.0

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

The results obtained, concerning the relation between the degree of the nasal histamine reactivity and the reaction of the nasal mucosa to moulds in various subgroups of the two age groups, are gathered in table 68.

For the statistical analysis, table 68 has been contracted into table 69.

Table 69

The comparison of the degree of the nasal histamine reactivity in the subgroups of the two age groups (for explanation of the subgroups, see table 63).

	≦ 40 years of age Subgroup					> 40 years of age Subgroup			
	1	2	3	4	5	1	2	4	5
Nasal complaints	+	+	+	+	—	+	+	+	—
Intracutaneous test to moulds	+	+	+	—	—	+	+	—	—
CNSLD	+	+	—	+	—	+	+	+	—
Nasal provocative test	+	—	—	—	—	+	—	—	—
N_{HR}									
≧ —1	5	13	3	13	6	0	16	9	4
≦ —2	10	5	0	5	0	2	5	3	0

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

Conclusion

1. The degree of the nasal histamine reactivity was found to be increased in the subjects with positive nasal provocative tests in comparison with those with negative nasal provocative tests (subgroup 1 versus 2). This difference is statistically, just insignificant — probably due to the small numbers represented here.
2. No clear "age effect" was found with respect to the relation between the nasal histamine reactivity and the reaction of the nasal mucosa to moulds. Likewise, no clear effect of CNSLD was found. However, the numbers of the subjects represented in the subgroups are very small.

C. The relation between the reaction of the nasal respiratory mucosa and the bronchial tree to moulds.

A survey of the nasal and bronchial provocative tests accomplished in a number of subjects is given in the next table.

Table 70

The results of the nasal and bronchial provocative tests with moulds in the various subgroups under and over 40 years of age. The subgroups are characterized by the presence (+) or absence (—) of:

— nasal complaints
— positive intracutaneous tests to moulds
— CNSLD

		Under 40 years of age Subgroup								Over 40 years of age Subgroup							
		1		2		3		4		1		2		4			
Nasal complaints		+		+		+		—		+		+		—			
Intracutaneous test to moulds		+		—		+		—		+		—		—			
CNSLD		+		+		—		—		+		+		—			
		AL_B		AL_B		AL_B		AL_B		AL_B		AL_B		AL_B			
		+	—	+	—	+	—	+	—	+	—	+	—	+	—		
AL_N	+	9	6	0	0	0	0	0	0	2	0	0	0	0	0		
	—	1	9	0	8	0	3	0	5	3	8	0	4	0	4		

AL_N: Nasal provocative test, positive (+) or negative (—)
AL_B: Bronchial provocative test, positive (+) or negative (—)

Conclusion

1. A positive nasal provocative test with moulds was found to occur only in subjects with nasal complaints, positive intracutaneous tests to moulds and with CNSLD. Likewise, a positive bronchial provocative test with moulds was found to occur only in subjects with positive intracutaneous tests to moulds and with CNSLD (subgroup 1).
2. An association was found between the bronchial tree and nasal mucosa with respect to the positive and negative bronchial and nasal provocative tests in subgroup 1, under 40 years of age (for explanation of the characteristics of this subgroup — see table 70.
3. In contradistinction to the relation between positive and negative nasal provocative tests with moulds in the two age groups, the relation between the positive and negative bronchial provocative tests with moulds in the two age groups was found to remain fairly constant (see table 71).

Table 71

Comparison between the positive (+) and negative (—) bronchial provocative tests with moulds in the two age groups.

		≦ 40 years of age	> 40 years of age
Bronchial provocative test	+	10	5
	—	15	8

IV. *Epidermals*

A. The relation between the result of the nasal provocative test and the clinical condition (presence or absence of nasal complaints).

A survey of the results of the nasal provocative tests with epidermals, is given in table 72.

Table 72

A representation of the positive and negative nasal provocative tests with epidermals, arranged according to:
— the presence or absence of nasal complaints
— the presence or absence of positive intracutaneous tests to epidermals
— age: under and over 40 years
— the presence or absence of CNSLD

Nasal complaints	Intracutaneous test to epidermals (i.c.)		Age groups	CNSLD positive Nasal provocative test		CNSLD negative Nasal provocative test	
				Positive	Negative	Positive	Negative
Positive	i.c.	+	≦ 40	9	26	0	4
			> 40	3	21	0	0
		—	≦ 40	0	19	0	0
			> 40	0	17	0	0
Negative	i.c.	+	≦ 40	0	0	0	0
			> 40	0	0	0	0
		—	≦ 40	0	0	0	12
			> 40	0	0	0	5

Conclusion

1. A positive nasal provocative test with epidermals was found to occur only in subjects with nasal complaints, positive intracutaneous tests to epidermals and with CNSLD.
2. In comparison with the results obtained in the provocative experiments done with grass pollen, house dust and moulds, less positive nasal provocative tests were found with epidermals.
3. No clear age or CNSLD effect on the results of the nasal provocative tests was found.

B. The relation between the nasal histamine reactivity and the reaction of the nasal mucosa to moulds.

The results concerning this relation, in various subgroups of the two age groups, are represented in table 73.

Table 73

The results of the investigation concerning the relation between the nasal histamine reactivity and the reaction of the nasal mucosa to epidermals in the two age groups. The subgroups are arranged according to the degree of the nasal histamine reactivity. For the explanation of the characteristics of the subgroups, see table 63.

	Under 40 years of age Subgroup					Over 40 years of age Subgroup			
	1	2	3	4	5	1	2	4	5
Nasal complaints	+	+	+	+	—	+	+	+	—
Intracutaneous test to epidermals	+	+	+	—	—	+	+	—	—
CNSLD	+	+	—	+	—	+	+	+	—
Nasal provocative test	+	—	—	—	—	+	—	—	—
N_{HR}									
6	0	0	0	0	3	0	0	0	2
5	0	1	0	1	4	0	0	0	2
4	0	0	0	1	2	0	0	0	1
3	0	7	0	0	3	0	5	3	0
2	0	1	1	1	0	0	2	2	0
1	0	3	0	0	0	0	1	4	0
0	0	3	0	4	0	0	8	2	0
—1	2	6	2	8	0	2	4	3	0
—2	2	3	1	3	0	1	1	2	0
—3	5	2	0	1	0	0	0	1	0
Mean	—2.3	+0.5	—0.5	—0.3	+4.6	—1.3	+0.7	+0.4	+5.2

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

For the statistical analysis table 73 has been contracted into table 74.

Table 74

The comparison of the degree of the nasal histamine reactivity in the subgroups of the two age groups (for explanation of the subgroups, see table 63).

	≦ 40 years of age Subgroup					> 40 years of age Subgroup			
	1	2	3	4	5	1	2	4	5
Nasal complaints	+	+	+	+	—	+	+	+	—
Intracutaneous test to epidermals	+	+	+	—	—	+	+	—	—
CNSLD	+	+	—	+	—	+	+	+	—
Nasal provocative test	+	—	—	—	—	+	—	—	—
N_{HR}									
≧ —1	2	21	3	15	12	2	20	14	5
≦ —2	7	5	1	4	0	1	1	7	0

N_{HR}: Nasal histamine reactivity expressed as x of 2^x mg/ml.

Conclusion

1. The degree of the nasal histamine reactivity was found to be significantly increased in subjects with nasal complaints, positive intracutaneous tests to epidermals, CNSLD and positive provocative tests to epidermals in comparison with the "non-responders" (subgroup 1 versus subgroup 2).
2. No clear age or CNSLD effect was found on the abovementioned relation.

C. The relation between the reaction of the nasal respiratory mucosa and the bronchial tree to epidermals.

A survey of the nasal and bronchial provocative tests achieved in a number of subjects is summarized in table 75.

Table 75

The results of the nasal and bronchial provocative tests with epidermals in the various subgroups under and over 40 years of age. The subgroups are characterized by the presence (+) or absence (—) of:
— nasal complaints
— positive intracutaneous tests to epidermals
— CNSLD

	≤ 40 years of age Subgroup				> 40 years of age Subgroup		
	1	2	3	4	1	3	4
Nasal complaints	+	+	+	—	+	+	—
Intracutaneous test to epidermals	+	+	—	—	+	—	—
CNSLD	+	—	+	—	+	+	—

AL_N	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —	AL_B +	AL_B —
+	5	4	0	0	0	0	0	0	1	2	0	0	0	0
—	1	13	0	4	0	8	0	5	2	5	0	5	0	4

AL_N: Nasal provocative test, positive (+) or negative (—)
AL_B: Bronchial provocative test, positive (+) or negative (—)

Conclusion

1. The finding, that a positive nasal provocative test, occurs only in a subject with nasal complaints, a positive intracutaneous test to the allergen tested (e.g. pollen, house dust, moulds), and with CNSLD — holds true for epidermals too. The same was found for the bronchial provocative test, namely, a positive bronchial

provocative test with epidermals occurred only in those subjects with positive intracutaneous tests to epidermals and with CNSLD (subgroup 1).

2. An association was found between the bronchial tree and nasal mucosa with respect to the positive and negative bronchial and nasal provocative tests in subgroup 1.
3. The relation between the positive and negative bronchial provocative tests was found to remain fairly constant for the epidermals too (see table 76).

Table 76

Comparison between the positive (+) and negative (—) bronchial provocative tests with epidermals in the two age groups.

		≤ 40 years of age	> 40 years of age
Bronchial provocative test	+	6	3
	—	17	7

Tabe 77

The results of the investigation concerning the relation between the degree of the nasal and bronchial histamine reactivity in the various subgroups under and over 40 years of age. The subgroups are characterized by the presence (+) or absence (—) of: nasal complaints (or manifest pollinosis) (+ or —) intracutaneous tests (+ or —); CNSLD (+ or —); positive nasal provocative tests (+ or —) and positive bronchial provocative tests (+ or —).

Number of subgroup		1			2			3	
Nasal complaints and manifest pollinosis		+			+			+	
Intracutaneous test		+			+			+	
CNSLD		+			+			+	
Nasal provocative test		+			+			—	
Bronchial provocative test		+			—			+	
		B_{HR}			B_{HR}			B_{HR}	
		≤ 2	≥3		≤2	≥3		≤1	≥
Under 40 years of age	≤—3	27	12	≤—3	4	3	≤—2	0	
	N_{HR}			N_{HR}			N_{HR}		
	≥—2	9	18	≥—2	7	7	≥—1	2	
Significance		$\chi_1 = 6.814$ $p = 0.01$			n.s.			n.s.	
		B_{HR}			B_{HR}			B_{HR}	
		≤2	≥3		≤2	≥3		≤3	≥
Over 40 years of age	≤—2	7	0	≤—2	1	0	≤ 0	4	
	N_{HR}			N_{HR}			N_{HR}		
	≥—1	3	5	≥—1	2	4	≥ 1	3	
Significance		n.s.			n.s.			n.s.	

§ 4. The relationship between the degree of the histamine reactivity of the nasal respiratory mucosa and the bronchial tree

As has been stated in the introduction, one of the objectives of the investigation described in this thesis, is the relationship between the reaction pattern of the nasal respiratory mucosa and the bronchial tree to non-antigenic stimuli.

If a comparable reaction may exist between the nasal mucosa and the bronchial tree to histamine, then it is conceivable that a relationship may also exist between the degree of the nasal and the bronchial histamine reactivity, in a group of persons with nasal and chest complaints.

Since the nasal and the bronchial reactivity are influenced by various factors (e.g. age, manifest allergy) homogeneous subgroups

N_{HR}: Nasal histamine reactivity
B_{HR}: Bronchial histamine reactivity
} expressed as x of 2^x mg/ml
n.s. : Statistically insignificant.

4

		B_{HR}	
		≤ 1	≥ 3
N_{HR}	≤ -1	12	12
	≥ 0	10	11

n.s.

5

		B_{HR}	
		≤ 3	≥ 4
N_{HR}	≤ -2	6	6
	≥ -1	8	7

n.s.

6

		B_{HR}	
		≤ 4	≥ 5
N_{HR}	≤ -1	8	8
	≥ 0	7	5

n.s.

7

		B_{HR}	
		≤ 5	≥ 6
N_{HR}	≤ -1	1	12
	≥ 0	2	6

n.s.

8

		B_{HR}	
		≤ 5	≥ 6
N_{HR}	≤ 3	2	8
	≥ 4	1	13

n.s.

		B_{HR}	
		≤ 1	≥ 2
N_{HR}	≤ 0	5	6
	≥ 1	5	6

n.s.

		B_{HR}	
		≤ 1	≥ 2
N_{HR}	≤ -1	6	6
	≥ 0	5	5

n.s.

		B_{HR}	
		≤ 2	≥ 3
N_{HR}	≤ -1	5	3
	≥ 0	2	2

n.s.

		B_{HR}	
		≤ 5	≥ 6
N_{HR}	≤ 4	1	5
	≥ 5	0	10

n.s.

were arranged for the analysis of this possible relationship. No subdivision was made according to the different allergens (viz. pollen or house dust or moulds or epidermals) as the numbers of individuals represented in the various subgroups, are very small.

A survey of the results is represented in the next table (table 77).

Conclusion

From the results presented in table 77, a statistically significant relationship between the degree of the nasal and the bronchial histamine reactivity, was found only in subgroup 1 under 40 years of age characterized by the presence of: nasal complaints (and manifest pollinosis), positive intracutaneous tests, CNSLD, positive nasal and bronchial provocative tests.

For a possible explication of these findings, the reader is referred to chapter VIII (General Discussion).

EXPERIMENTAL INVESTIGATION REGARDING THE EFFECT OF NON-SPECIFIC (NON-ALLERGIC) STIMULI ON THE NASAL RESPIRATORY MUCOSA

§ 1. Introduction

Non-specific nasal reactions can be defined as those reactions which occur as the result of a non-allergic mechanism by physical and chemical agents, with the exception of reactions due to drugs.

Experiments done with such non-specific stimuli have already been mentioned in chapter II. More recently, SPEIZER and FRANK (1966) reported experiments in which healthy males were exposed to sulphur dioxide by nose and by mouth.

In this chapter, the results of some provocative experiments with non-specific stimuli, namely, ammonia and sulphur dioxide, will be given. In each person included in this study, the histamine reactivity of the nasal respiratory mucosa was first established. These subjects were selected from those attending the Pulmonary Division of the Department of Medicine, State University Hospital, Groningen.

§ 2. Experiments with sulphur dioxide (SO_2)

The effect of SO_2 on the nasal respiratory mucosa was studied in 10 patients. The same method as has been described in chapter III for histamine, was used. The following concentrations of freshly prepared sulphur dioxide* were applied, namely 0.03, 0.06, 0.12, 0.24 and 0.48 %. The results of these experiments are summarized in table 78.

In another trial, the response to nebulized SO_2 was assessed simultaneously in the bronchial tree and nasal respiratory mucosa. The same method was used, as has been described for the simultaneous determination of the reaction of the nasal respiratory mucosa and

* prepared by an iodometric method.

Table 78

A comparison of the effect of histamine and sulphur dioxide on the nasal respiratory mucosa in 10 patients with nasal and chest complaints.

No.	Age	Sex	Nasal complaints	CNSLD	Predicted value VC	Predicted value FEV_1	Measured value VC	Measured value FEV_1	B_{HR}	N_{HR}	P_I	P_{II}	SO_2 conc. in %	Observation of nasal reaction* Histamine O	Histamine N	Histamine S	SO_2 O	SO_2 N
1	25	M	+	+	6500	5005	3800	1675	1	—3	5.6	5.6	0.48	+++	—	+	—	—
2	20	M	+	+	4270	3416	4000	2725	3	—2	4.2	4.2	0.48	+++	—	+	—	—
3	17	F	+	+	3750	2819	3100	2600	5	—2	5.6	5.6	0.48	+++	—	—	—	—
4	25	M	+	+	4180	3219	3225	1625	1	—1	2.8	2.8	0.48	+++	—	—	—	—
5	21	F	+	+	3520	2816	3100	2150	5	—3	7.6	7.0	0.48	+++	—	+	—	—
6	18	F	+	+	4400	3250	3500	2650	0	2	8.0	8.4	0.48	+++	—	+	—	—
7	43	M	+	+	5320	4670	3025	1175	2	—3	3.5	3.5	0.48	+++	—	+	—	—
8	20	F	+	+	3885	2991	3425	2000	1	—3	6.2	6.0	0.48	+++	+	+	—	—
9	16	F	+	+	3685	2991	2925	1850	1	—3	5.6	5.6	0.48	+++	—	+	—	—
10	43	M	+	+	4700	3713	4770	3625	5	4	2.8	2.8	0.48	++	—	+	—	—

* For interpretation of the reaction see chapter V, § 2.

N_{HR} : Nasal histamine reactivity
B_{HR} : Bronchial histamine reactivity
} expressed as x of 2^x mg/ml

P_I : Initial pharynx-nostril pressure gradient in cmH_2O before topical application of SO_2

P_{II} : Pharynx-nostril pressure gradient in cmH_2O after topical application of SO_2

VC : Vital Capacity

FEV_1 : Forced Expiratory Volume in one second.

Table 79

A comparison of the effect of histamine and nebulized sulphur dioxide on the nasal respiratory mucosa in 10 patients with nasal and chest complaints. This table also shows a comparison of the nasal and bronchial reaction to sulphur dioxide and some simple lung function data of these patients.

			Predicted value		Measured value		VC and FEV_1 in % of predicted value							Observation of nasal reaction*					
														Histamine			SO_2		
No.	Age	Sex	VC	FEV_1	VC	FEV_1	VC	FEV_1	B_{HR}	N_{HR}	P_I	P_{II}	B_{SO_2} in %	O	N	S	O	N	S
1	20	F	4610	3549	3850	2800	84	79	1	—3	5.6	7.0	>0.48	++±	—	+	—	—	—
2	50	M	4750	3088	4200	1975	88	64	2	—1	4.2	4.8	0.48	++	—	—	+	—	++
3	16	M	2830	2264	2565	2012	91	89	4	—3	5.6	6.3	0.03	++	—	—	—	—	—
4	14	M	4270	3416	3540	1862	83	55	—1	—3	8.4	8.4	0.06	++	—	—	—	—	—
5	17	M	4130	3304	3525	2165	85	66	—1	—3	4.2	9.8	0.06	+++	—	—	+	—	+±
6	17	M	5040	3880	4100	2600	81	67	—1	—1	5.6	7.7	0.06	+++	—	—	—	—	—
7	19	M	5200	4004	4140	3465	80	87	4	—2	4.9	5.6	>0.48	++	—	—	—	—	—
8	45	M	3775	1650	3912	1375	104	83	1	—2	4.9	3.5	0.24	+++	—	+	—	—	+
9	49	M	4060	3150	3192	1250	79	40	—1	0	4.2	3.2	0.12	++±	—	—	—	—	+±
10	57	M	4180	2717	4587	2412	110	89	0	—1	5.6	5.8	0.48	++	—	—	—	—	+

* For interpretation of the reaction see chapter V, § 2.

N_{HR} : Nasal histamine reactivity
B_{HR} : Bronchial histamine reactivity
} expressed as x of 2^x mg/ml

P_I : Initial pharynx-nostril pressure gradient in cmH_2O before nebulization of SO_2

P_{II} : Pharynx-nostril pressure gradient in cmH_2O after nebulization of SO_2

VC : Vital Capacity

FEV_1: Forced Expiratory Volume in one second

B_{SO_2}: Bronchial threshold value to SO_2 (a decrease of the VC and/or FEV_1 of 10 % or more)

Table 80

A comparison of the effect of histamine and ammonia on the nasal respiratory mucosa in 20 patients with nasal and chest complaints.

No.	Age	Sex	Nasal complaints	CNSLD	Predicted value VC	Predicted value FEV_1	Measured value VC	Measured value FEV_1	B_{HR}	N_{HR}	P_I	P_{II}	"2 P_I" %	Observation of nasal reaction* Histamine O	Histamine N	Histamine S	Ammonia O	Ammonia N	Ammonia S
1	20	M	+	+	5550	4274	4450	2750	1	—2	5.6	12.6	0.256	+++	—	+	+	+	++
2	21	M	+	+	5520	4130	5175	4400	5	—4	7.0	26.0	0.064	+++	+	++	++	—	+++
3	35	M	+	+	4925	3595	4950	3600	4	—2	8.4	17.0	0.064	+++	—	+	+	+	+++
4	20	F	+	+	4350	3480	4050	3200	2	—3	2.1	8.4	0.128	+++	+	+	+	—	++
5	34	F	+	+	3390	2475	2975	2850	6	1	5.6	10.0	1.024	++	—	+	±	—	+
6	60	M	+	+	4165	2707	3800	2300	2	4	2.8	7.0	1.024	++	—	+	±	—	±
7	30	M	+	+	4850	3541	4525	3300	4	1	4.2	10.8	0.512	+++	—	—	±	—	+
8	36	F	+	+	3610	2635	2550	1587	2	—1	7.0	18.2	0.256	+++	—	+	+	—	+
9	17	M	+	+	4480	3584	3825	2800	5	—3	8.4	19.6	0.128	+++	—	+	+	—	++
10	54	F	+	+	3550	2475	3000	2325	6	3	2.8	4.2	1.024	++	—	—	±	—	+
11	43	M	+	+	5320	4670	3025	1175	2	—3	3.5	9.0	0.128	+++	—	+	+	—	++
12	22	M	+	+	5395	4150	5375	4350	4	—3	2.5	9.0	0.256	+++	+	+	±	—	++
13	13	M	+	+	4000	3200	3300	2275	4	—3	4.5	12.6	0.064	+++	++	++	+±	+	++
14	17	M	+	+	4250	3300	3850	1850	0	—3	3.0	7.0	0.128	+++	+	+	+	—	+
15	22	M	+	+	4880	3700	3187	1775	0	—3	7.5	20.0	0.064	+++	+	+	+	+	++
16	55	M	+	+	3605	2343	2950	2125	1	1	8.6	16.0	0.512	++	—	+	±	—	+
17	45	M	+	+	4150	2864	4350	2700	2	—2	5.6	15.0	0.128	+++	—	+	+	—	++
18	33	M	+	+	4550	3650	3825	2850	3	1	3.0	12.6	0.512	++	—	—	±	—	++
19	46	F	+	+	3420	2360	2175	1500	4	2	6.0	13.0	0.512	+++	—	—	±	—	+
20	45	M	+	+	4250	3250	3800	2075	5	2	7.3	16.0	0.512	++	—	+	±	—	+

* For interpretation of the reaction see chapter V, § 2.

N_{HR} : Nasal histamine reactivity
B_{HR} : Bronchial histamine reactivity
} expressed as x of 2^x mg/ml

P_I : Initial pharynx-nostril pressure gradient in cmH_2O before topical application of ammonia solutions

P_{II} : Pharynx-nostril pressure gradient in cmH_2O after topical application of ammonia solutions

"$2P_I$": See chapter III, § 4

VC : Vital Capacity

FEV_1: Forced Expiratory Volume in one second.

Table 81

A comparison of the reaction of the nasal respiratory mucosa to histamine and ammonia in 20 patients with nasal and chest complaints.

		Nasal histamine reactivity expressed as x of 2^x mg/ml	
		$\leqq -2$	$\geqq -1$
Ammonia in %	$\leqq 0.128$	9	0
	$\geqq 0.256$	2	9

bronchial tree to nebulized histamine (see chapter III, § 6). These results are summarized in table 79.

Conclusion

1. Topical application of SO_2 had no effect on the nasal respiratory mucosa in patients with nasal and chest complaints, while on the contrary, topical application of histamine caused pronounced effects.

2. In a number of patients (5 out of 10) with nasal and chest complaints, and an increased reactivity of the nasal respiratory mucosa to histamine, nasal hypersecretion sometimes accompanied by a slight mucosal swelling (2 patients in this study — see table 79) occurred following the nebulization of SO_2. Furthermore, severe bronchial obstruction occurred in 8 patients (total: 10) with an increased reactivity of the bronchial tree to histamine.

§ 3. Experiments with ammonia solutions on the nasal respiratory mucosa

The effect of ammonia on the nasal respiratory mucosa was studied in 20 patients. Mounting concentrations of ammonia* were applied to the nasal respiratory mucosa by using the same method as in the case of histamine provocation (see chapter III). The concentrations were: 0.008, 0.016, 0.032, 0.064, 0.128, 0.256, 0.512 and 1.024 %.

A survey of these results, is represented in table 80 and 81.

Conclusion

1. Topical application of ammonia to the nasal respiratory mucosa

* Prepared by titration with HCl.

in patients with nasal and chest complaints under and over 40 years of age, caused mainly nasal hypersecretion and, to a minor degree, a swelling of the mucosa. Histamine on the other hand, caused a pronounced swelling of the nasal respiratory mucosa accompanied by a slight nasal hypersecretion.

2. A statistically significant relationship was found between the degree of the nasal histamine and -ammonia reactivity.

DISCUSSION AND CONCLUSION OF THE INVESTIGATION CONCERNING THE REACTIVITY OF THE NASAL RESPIRATORY MUCOSA

§ 1. Practical problems in the achievement of the reactivity of the nasal respiratory mucosa

The reactivity of the nasal respiratory mucosa can be considered as the central theme of this thesis. It is defined as the lowest concentration of histamine causing a change in the pharynx-nostril pressure gradient, twice the initial pressure value (see chapter III, § 4).

This working definition was chosen as a starting point for discusion since a close analysis of this definition will reveal the different problems involved, in a logical manner.

This definition is based on the following suppositions.

a. Histamine can be regarded to be representative for other possible stimuli causing a reaction in the nasal mucosa.
b. Respiratory mucosal changes in the nose are always accompanied by changes in the nasal passage.
c. The parameter applied in the assessment of the nasal passage is most suitable for this investigation.
d. The results are not influenced by the technique applied.

These suppositions cannot simply be accepted and therefore, some elaboration is needed.

ad a. Whether histamine can be considered to be representative for other stimuli has to be confirmed by further investigation. However, in this investigation, histamine was used as the "model agent"; therefore, the effects of other stimuli were all compared with the effects induced by histamine.

ad b. In chapter III, § 3, it was clearly demonstrated that a change in the nasal passage is accompanied by a swelling of the nasal mucosa. In this regard however, a problem lies in the interpretation of the notion — "changes of the nasal mucosa", since a change of the mucosa does not necessarily imply to be identical with a swelling of the mucosa. Changes, as for instance hypersecretion, may also indicate changes of the nasal mucosa; however, it does not necessarily indicate a narrowing of the nasal passage as in the case of a swelling of the mucosa with subsequent increase of the pharynx-nostril pressure gradient.

In view of the clinical importance of changes in the nasal passage, e.g. nasal obstruction, this restriction is not considered as a disadvantage. However, a more fundamental description is required for the conception "reactivity of the nasal respiratory mucosa". Attention will be paid to this problem.

ad c. As a parameter for changes in the nasal passage, a certain degree of alteration in the pharynx-nostril pressure pradient has been employed (chapter III, § 4).

The pharynx-nostril pressure gradient is related to the velocity of air flow through the nasal chambers. Variations of the velocity of air flow (different "flow rates"), without changes in the nasal chamber(s) [e.g. no swelling of the mucosa], are associated with changes in the pharynx-nostril pressure gradient. Thus, even though the calibre of the nasal chambers remains constant, different values for the pharynx-nostril pressure gradient will be obtained for different, "flow rates", and clinically, "flow rates", may vary from one respiration to another.

In this investigation, the volume of air through one nasal chamber, as well as the pharynx-nostril pressure gradient (see chapter III, § 3), were registered simultaneously during normal respiration.

From the results obtained by comparison of the different parameters (chapter III, § 4), it appears that a change in the pharynx-nostril pressure gradient twice the initial value, following the application of histamine, is accompanied by a change in the nasal passage (observed as being mainly a swelling of the mucosa). This criterion seems therefore acceptable in the assessment of the nasal

passage. A further argument for the applicability of this simple technique and for the criterion ($2P_I$) for the assessment of the nasal passage, is found in the demonstration that the nasal passage was assessed in a simple but objective way, in a large group of patients, as well as in epidemiological groups (as described in the previous chapters).

ad d. Possible sources of error, which can be considered to be involved in the assessment of the nasal histamine reactivity, e.g. the influence of mechanical irritation and the influence of the solvent, have no effect on the results obtained (chapter III, § 5).

Influence of the anatomical structure on the nasal histamine reactivity

The nasal histamine reactivity is influenced by the anatomical structure in subjects with a distinct deviation of the nasal septum (chapter III, § 6). An increased susceptibility of the nasal mucosa to histamine exists at the side towards which the septum is deviated and, there is also an increased pharynx-nostril pressure gradient. This relationship between the initial pharynx-nostril pressure gradient and the histamine reactivity of the nasal respiratory mucosa, may furnish an argument against the applicability of the working definition of the nasal histamine reactivity. In this regard, however, the initial pharynx-nostril pressure gradient can probably be taken as an indicator for a certain nasal anatomical structure with a certain clinical syndrome. The problem regarding the initial pharynx-nostril pressure gradient, extends the discussion to the problem concerning the relationship between certain clinical syndromes and the reactivity of the nasal respiratory mucosa, which is described in the next paragraph.

§ 2. The problem of the relationship between the reactivity of the nasal respiratory mucosa and the clinical picture

The design for an investigation concerning the relationship between the reactivity of the nasal respiratory mucosa and clinical syndromes, has an epidemiological character. In the case of such an investigation, selection of subjects must be avoided, which implies an investigation on the basis of samples taken at random from a "normal" po-

pulation. On this basis, an investigation was performed in two epidemiological groups (see chapter IV).

The histamine reactivity of the nasal respiratory mucosa had to be assessed in about 2 × 100 subjects within a period of 2 × 1 week. Therefore, a reduction in the number of histamine concentrations (as described in chapter III, § 3), was necessary. This reduction, however, has no influence on the principle of the assessment of the reactivity.

Description of the clinical syndrome

Two possible approaches can be considered in defining a "clinical syndrome" in such an investigation:

a. the reactivity of the nasal mucosa can be assessed at random by the investigator, who at the same time inquires about nasal complaints in order to obtain a clinical diagnosis;
b. the nasal histamine reactivity can be assessed at random by the investigator from a randomly chosen group of subjects in whom the nasal complaints were recorded by trained medical staff members, using a standardised questionnaire.

Since an independent assessment of the reactivity of the nasal mucosa and of the nasal complaints cannot be obtained by the first approach, the second approach seems most suitable, and was chosen. For obvious reasons the epidemiological survey including 2 × 1000 individuals, the characterization of the nasal complaints — had to be simple.

The epidemiological "picture" of nasal complaints, obtained from these subjects over 40 years of age, can be summarized as follows.

a. The difference in prevalence of nasal complaints as compared with data from the literature, although not significant, is probably due to the use of different questionnaires.
b. Over 40 years of age, no "age pattern" concerning the complaints exists, which is in accordance with the literature.
c. Generally, a predominance of males with nasal complaints exists. It is not clear on which mechanism this difference is based. It may be taken into account that males are more frequently exposed to exogenous stimuli than females. Furthermore, specific endogenous factors (hormonal) should be considered as a pos-

sible explanation for this sex difference. This is also in accordance with the literature.

d. Exogenous stimuli (e.g. industrial air pollution) probably play an important rôle in the manifestation of nasal complaints, since a significantly higher prevalence, both in males and females, is found in Vlaardingen than in Sellingen.
e. A significant correlation exists between nasal and chest complaints.

A general conclusion cannot be made from the data of this epidemiological study, because only subjects over 40 years of age were included. Furthermore, these data do not settle the question whether the combination of these nasal complaints corresponds with the clinical picture known as "vasomotor rhinitis", which is not uniformly described in the literature (see chapter II, § 2). It is highly probable that the nasal complaints are related to this condition.

Relationship between the histamine reactivity of the nasal respiratory mucosa and nasal complaints, in the epidemiological groups

A significant relationship exists between the nasal complaints and the histamine reactivity of the nasal respiratory mucosa in the two epidemiological groups. In an attempt to specify this relationship, the following possibilities should be considered:

a. an increased reactivity of the nasal respiratory mucosa is present, when exposure results in nasal obstruction, hypersecretion or sneezing (or a combination of these);
b. a swelling of the epithelium and nasal complaints co-exist, causing an increased reactivity of the nasal mucosa.
   A final conclusion from these two possibilities seems difficult, however, arguments in favour of the latter are
   — in subjects with a distinct deviation of the nasal septum, increased reactivity of the nasal mucosa, and increased initial pharynx-nostril pressure gradient co-exist at the narrowed side;
   — the finding that the histamine reactivity of the nasal mucosa, both the initial pharynx-nostril pressure gradient, and the

prevalence of nasal complaints are present in a higher degree in the epidemiological group of Vlaardingen than in the group of Sellingen.

However, since in both epidemiological groups no clear association was found between the initial pharynx-nostril pressure gradient and the histamine reactivity of the nasal respiratory mucosa, these arguments are not as yet conclusive. This dissociation can only be explained if other factors are also involved, disguising the relationship between the nasal histamine reactivity and the initial pharynx-nostril pressure gradient.

c. Finally, another possibility should be considered, i.e., nasal histamine reactivity and nasal complaints are influenced independently by an unknown factor.

Clinically, the conclusion can be made that an increased reactivity of the nasal repiratory mucosa to histamine is pathognomonic for nasal complaints (e.g. obstruction), as seen in "vasomotor rhinitis".

No sex effect exists in the prevalence of the degree of the nasal histamine reactivity; the same holds true in respect to an age effect in persons over 40 years of age. However, between the two random samples, a marked difference concerning the nasal histamine reactivity exists: the degree of the nasal histamine reactivity is found to be much higher (lower threshold values) in the epidemiological group of Vlaardingen than in the group of Sellingen, whereas the nasal complaints are not increased to the same degree. As has already been mentioned, a statistically significant difference exists between the initial pharynx-nostril pressure gradient of the two epidemiological groups.

It seems reasonable to explain the difference between the two epidemiological groups as the result of the influence of an "environmental factor". The precise nature of this factor(s) is unknown. Furthermore, the mechanism by which this "environmental factor" acts upon the nasal respiratory mucosa, cannot be inferred from the results of this study. The following explanation however, can be considered: the stimulus of the "environmental factor" acts in the same way as histamine; by means of this more or less continuous "natural" exposure to "mucosal active substances", less exogenous

histamine is necessary to cause a positive reaction as defined (chapter III, § 4) by using the criterion $2P_1$. The mean difference ($\pm$ 0.5 cmH_2O) of the initial pharynx-nostril pressure gradient between the two epidemiological groups may perhaps provide an argument in this respect, considering the fact that this value lies on the beginning of the steep part of the slope of the "S" — shaped dose-response curve. Moreover, this possibility is in accordance with the finding that no significant age effect exists on the degree of the nasal histamine reactivity over 40 years of age. An indication for a "direct alteration" in the effector organ by the "environmental factor" has not been found. It is conceivable that this "direct alteration" (whatever it may be) could have reached its maximum before the age of 40 years, with the result that an age effect cannot be demonstrated over 40 years.

The age factor is more or less an indication as to the duration of the exposure to the stimuli. A closely related problem in this regard is the existence of a "pure age effect" on the degree of the histamine reactivity of the nasal respiratory mucosa. To analyse this problem, the degree of the nasal histamine reactivity was compared in normals both under and over 40 years of age; the older age group (clinical controls) was also compared with data from the epidemiological groups (subjects without nasal complaints). The results are summarized in table 82.

Table 82

A comparison of the degree of the nasal histamine reactivity in the control groups ($\leq$ 40 and $>$ 40 years) of the patient group, with "normals" from two epidemiological groups.

	Control groups		Epidemiological groups	
			Sellingen	Vlaardingen
N_{HR}	$\leq$ 40 years	$>$ 40 years	($\geq$ 40 years)	($\geq$ 40 years)
5	9	5	53	19
4 & 5	20	11	1	3
2 & 3	9	1	4	5
0 & 1	2	1	3	11
—3 & —1	4	0	0	2

N_{HR}: Histamine reactivity of the nasal respiratory mucosa expressed as x of 2^x mg/ml.

A significant difference exists between the clinical group over 40 years of age and the random sample from Sellingen with respect to the degree of the nasal histamine reactivity. Therefore, the

"normals" from the clinical group cannot be taken to be representative for "normals" of the Sellingen group; the same holds true for the "normals" of the Vlaardingen group.

The degree of the nasal histamine reactivity of the "normals" of the clinical group are in between the "normals" of Sellingen and Vlaardingen. Although no significant difference in the degree of the nasal histamine reactivity could be demonstrated between the two age groups (under and over 40 years) in the "normals" of the clinical group, still this reactivity tends to be higher in the "normals" under 40 years of age. The age factor (age confined factors) cannot be analysed further from the data of the epidemiological surveys. An attempt will therefore be made to analyse this problem in the clinical group of patients; these results are shown in the next table.

Table 83

The influence of age on the degree of the histamine reactivity of the nasal respiratory mucosa in patients with nasal complaints, intracutaneous tests positive (+)or negative (—), CNSLD, and positive (+) or negative (—) nasal provocative tests (the results are pooled).

	Subgroup					
	1	2	3	4	5	
Nasal complaints	+	+	+	+	+	
Intracuteaneous test	+	+	—	—	+	
CNSLD	+	+	+	—	—	
Nasal provocative test	+	—	—	—	—	
	≤ 40 yrs	> 40 yrs	≤ 40 yrs	> 40 yrs		
N_{IIR} ≥ —2	39	18	≥ 0	69	76	
N_{IIR} ≤ —2	48	4	≤ —1	114	47	
	$\chi_1^2 = 8.217$		$\chi_1^2 = 16.126$			

N_{IIR}: Histamine reactivity of the nasal respiratory mucosa expressed as x of 2^x mg/ml.

A significant "age effect" on the histamine reactivity of the nasal respiratory mucosa exists in patients with nasal complaints, viz. the nasal histamine reactivity is found to be higher (lower threshold values) in subjects with nasal complaints under 40 years of age than in those over 40 years of age. An explanation of this difference renders some difficulties: since the results of the intracutaneous tests in the subgroups do not provide an argument for an age-dependent, allergic factor. If, furthermore, the duration of the intensity of exposure to exogenous factors should play an im-

portant rôle, then the opposite result should be obtained (increasing reactivity with old age). It is not obvious how this difference should be interpreted. Probably it is linked with intrinsic changes in the effector organ. An epidemiological investigation of subjects under 40 years of age concerning nasal complaints and the degree of the histamine reactivity of the nasal respiratory mucosa, may clarify the issue.

§ 3. Interpretation of the results regarding the effects of pharmacological and chemical stimuli on the nasal respiratory mucosa

In view of the histological structure of the nasal respiratory mucosa, it is most likely that the "process of reactivity" is the result of *vascular reactivity:* either by constriction or dilatation in different regions of the nasal vascular system, accompanied by or leading to changes in the vascular permeability. Another site of action of stimuli may be in the nasal mucous glands and goblet cells (hypersecretion) which, however, play a minor rôle in the reactivity of the nasal mucosa.

Some clinical experiments were performed with the object to acquire more information concerning the nature and localization of the process of the nasal "hyperreactivity". In the next table a concise resumé is represented concerning the actions of different substances on the blood vessels and mucous glands both from the literature and personal experiments. From the literature it appears, that in some instances qualitative as well as quantitative differences exist with respect to the action of these agents.

It must be pointed out that in these clinical experiments the *observed swelling of the nasal mucosa was always accompanied by an increase in the pharynx-nostril pressure gradient.*

Acetylcholine

One of the most striking findings of these experiments, is the difference of effect between histamine and acetylcholine on the nasal mucosa as represented in chapter V, § 2. These findings suggest that the phenomenon of reactivity, as defined in this thesis, is based on the *contraction of arterioles* and *venules;* a primary disturbance in the permeability, is unlikely.

Table 84

A representation of selective effects of pharmacological and chemical agents on the vascular system according to the literature and own observation.

	Literature: Vascular system					Own observation*		
	Arterioles	Precapillary sphincters	Venules	Increase in capillary permeability	Increase in mucous glandular secretion	O	S	N
Histamine	C	D	C	+	+	+ + +	+ ±	+
Acetylcholine	D	C	D	?	+	±	+ +	—
5-Hydroxytryptamine	C	D	C	+	?	—	—	—
Bradykinin	Dilatation (not specified)			+	?	—	—	—
Compound 48/80	Action by histamine release?			?	?	—	—	—
Isoproterenol	C	CC	C	?	?	+	+	—
Allergens	C	?	?	?	?	+ +	+ +	+
Ammonia	?	?	?	?	?	+	+ +	±
Sulphur dioxide	?	?	?	?	+	±	+	—

C: Constriction
D: Dilatation

O: Swelling of the mucosa
S: Nasal hypersecretion
N: Sneezing

* For interpretation of reaction see table 53.

5-Hydroxytryptamine

As stated before, histamine causes a pronounced change in the nasal mucosa; on the contrary, 5-hydroxytryptamine has no effect. It may be argued that the concentration of 5-hydroxytryptamine was too low; as however, a 2 % solution was applied, this explanation seems doubtful. The most probable explanation seems to be that, in contrast to histamine, no specific receptors for this substance exist in the nasal respiratory mucosa.

Bradykinin

From the literature (see table 84), bradykinin can be regarded as a vasodilating substance; no effect was observed however, by the application of this substance to the nasal mucosa. Perhaps in this case, the concentration was too low.

Compound 48/80

The reason(s) why no reaction occured following the administration of this compound may be due to:

— the local absence of endogenous histamine; however, in view of the literature this possibility seems very unlikely;
— inadequate concentration of this compound at the site of release, which, however, also seems doubtful;
— a too slow liberation of histamine by this compound in man (HALPERN);
— the protective effect of mast cells with certain histamine releasors, such as this compound [HIGGINBOTHAM (1966), see fig. 19].

In spite of substantial evidence, that histamine is released by this compound (see chapter V, § 5), it remains to be demonstrated which of these possibilities (or other possibilities) holds true in our case.

Isoproterenol

The action of isoproterenol (beta-receptors) on the vascular bed resembles that of histamine to a certain extent (except for the action on the precapillary sphincters), and may probably be fitted into the described basis phenomenon of the reactivity.

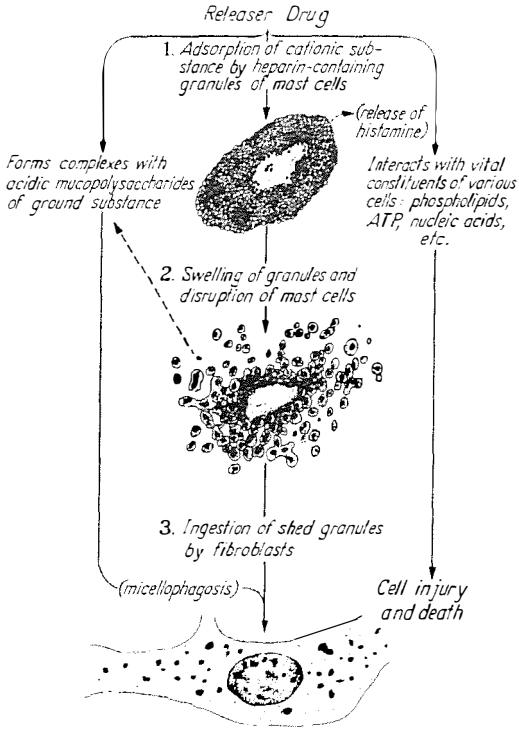


Fig. 19

Proposed mechanism of the protective aspects of mast cells with certain histamine-releasing agents, such as compound 48/80.

(According to Higginbotham, 1966).

Ammonia and sulphur dioxide

The mechanism(s) by which these substances act on the nasal respiratory mucosa is (are) unknown. The reaction pattern to a certain extent "resembles" that induced by acetylcholine. This reaction is probably based on a reflex (para-sympathetic stimulation). Reactions of the nasal respiratory mucosa, e.g. hypersecretion and swelling, seems to diverge. With reference to the way of assessing the reactivity of the nasal respiratory mucosa, it must be stressed again, that the investigation of the reactivity is mainly confined to a certain reaction (swelling) of the mucosa, which in all probability depends upon a *contraction of the smooth muscle cells of arterioles, venules, and sphincters at the end of the venous sinusoids.*

§ 4. Relationship between the reactivity of the nasal respiratory mucosa and the bronchial tree to allergens

Since the reactivity of the nasal respiratory mucosa is significantly increased (lower threshold values) in patients with positive nasal provocative tests with allergens, in comparison with "non-responders", an association exists with a manifest allergy. The character of this association is, however, not obvious.

It is conceivable that in the positive nasal provocative tests with house dust, moulds, and epidermals, the "natural" exposure is also involved; if we leave out the question of whether the allergic reaction acts via a direct action of the allergen-reagin complex itself on the vascular system, or via an indirect action by the release of histamine.

This does not apply, however, for patients with positive nasal pollen provocative tests, since these tests were not performed during the pollen season so that in this regard a "natural exposure" can be disregarded. Therefore, one should consider that the process of sensitization, e.g. the binding of reagin to the cell surface, has given rise to alteration in the reactivity of the contractile elements. Although this explanation may fit in the conception of TIFFENEAU, convincing arguments in favour of this possibility are lacking, since the skin histamine threshold value is not increased in allergic individuals as compared with non-allergic subjects. Another possibility to be considered, is that the positive nasal provocative tests can be explained by virtue of the increased histamine reactivity of the mucosa; if this is accepted, then it should be stressed that the increased nasal histamine reactivity co-exists with, but *independently* of the process of sensitization. To test this possibility, the degree of nasal histamine reactivity was compared in two groups of patients under 40 years of age, as shown in table 85.

Table 85

A comparison of the degree of the nasal histamine reactivity in patients with positive and negative nasal provocative tests to house dust, moulds and epidermals. All these patients had similar symptoms (see chapter VI).

		Provocative test	
		Positive	Negative
N_{HR}	≥ -1	13	57
	≤ -2	49	18

N_{HR}: Histamine reactivity of the nasal respiratory mucosa expressed as x of 2^x mg/ml.

A significant relationship exists between the degree of histamine reactivity of the nasal respiratory mucosa, and positive or negative provocative tests with allergens. In this regard it is assumed that the process of sensitization is actually the same in both groups on the basis of the intracutaneous tests. However, no threshold values of the intracutaneous tests were performed; and therefore this assumption cannot be confirmed quantitatively. Moreover, if the intracutaneous threshold values were comparable, this does not imply that the process of sensitization is identical. If this argument is not important, then it can be said that the positive allergen nasal provocative test is determined by the reactivity of the mucosa which, however, exists independently of the process of sensitization — meaning that the reactivity of the nasal mucosa is not solely determined by mechanical or exogenous factors.

Finally, the relationship between the reactivity of the nasal respiratory mucosa and of the bronchial tree will be considered. Theoretically these are hardly comparable, since:

a. the anatomical structure of the nose and bronchial tree is different;
b. the contractile elements in the bronchial tree are present in a higher degree than in the nose;
c. the vascular system of the nasal respiratory mucosa is different from that of the bronchial tree.

It is, therefore, conceivable that no definite correlation will exist in most of the subgroups as represented in chapter VI, § 4 with regard to the histamine reactivity. A quantitative relationship exists, however, between the reactivity of the nasal respiratory mucosa and the bronchial tree in patients with positive allergen provocative tests of nose *and* bronchial tree. An appropriate explanation could be that allergens play the most important rôle in the genesis of reactivity. A problem is that this explanation may hold true for the provocative tests with house dust, moulds and epidermals, but not for the pollen provocative tests.

It seems doubtful that this relationship (between the reactivity of the nasal respiratory mucosa and the bronchial tree) should be the result of the *selection* of the positive allergen provocative tests; it holds true for the increased degree of reactivity, but not for the

quantitative relationship of the reactivity of the nasal mucosa and the bronchial tree.

This dilemma, explained by *selection* or by a certain pathophysiological process, cannot be analysed further by the data obtained in this study.

Notwithstanding, the conclusion can be made that the *nasal respiratory mucosa shows a similar pattern of reaction to allergens as the bronchial tree.*

This does not necessarily mean that the (patho)physiological processes are identical. Furthermore, the *non-specific (non-allergic) reactivity of the nasal respiratory mucosa cannot simply be taken as a "model" for the reactivity of the whole respiratory tract.*

§ 5. Final remarks

It is clear that the conception "reactivity of the nasal respiratory mucosa" is an extreme simplification and formulation of the real physiological or pathophysiological processes in the nasal mucosa on reaction to stimuli. It is not intended to provide a more detailed physiological and biochemical evaluation. In fact, it still remains a problem whether *one* or more processes play a rôle, or whether *one* or more "reactivities" exist. A certain "variegated" conception of "reactivity" is necessary when the described results are surveyed.

The following factors should be considered in the interpretation of the degree of the histamine reactivity of the nasal respiratory mucosa.

1. *Mechanical factors*

A relationship exists between the histamine reactivity of the nasal respiratory mucosa and the initial pharynx-nostril pressure gradient in patients with an anatomical lesion, viz. septum deviation. However, the degree of reactivity cannot solely be inferred from this factor.

2. *Exogenous factors*

An intensified exposure to exogenous stimuli influences the degree

of the histamine reactivity of the nasal mucosa. This possibility seems very likely as was demonstrated by the comparative random investigations in Sellingen and Vlaardingen. However, in view of the results of other investigations, they are not the only determining factor(s).

3. *Age effect*

In patients with nasal complaints an age effect exists altering the degree of reaction of the nasal respiratory mucosa to histamine [this degree is found to be higher (lower threshold values) in individuals under 40 years of age than in those over 40 years].

4. *The number of smooth muscle elements in different parts of the vascular system*

Since no histological study was included in this investigation, no direct conclusions can be made concerning the rôle played by these elements.

5. *Increased susceptibility of the separate contractile elements*

This factor should perhaps be considered as the "essential" reactivity.

Clinically, it is important that (certain) nasal complaints are closely related with the degree of the histamine reactivity.

SUMMARY

The object of this investigation was to see whether the nasal respiratory mucosa reacts in a similar manner as the bronchial tree to exogenous stimuli. From a theoretically and practically point of view, the results could be of importance for a better evaluation of the reactivity of the lower respiratory tract.

Chapter I

In the first chapter, a description is given of the anatomy of the external and internal nose, the histology of the nasal mucous membrane and its nerve and blood supply. Attention is further paid to the respiratory functions of the nasal mucosa, the nasal reflex pathways and the pathophysiology of nasal obstruction, nasal secretion and sneezing.

Finally, a brief review of the literature is given, dealing with changes in the bronchial tree following upper respiratory tract irritation.

Chapter II

An attempt has been made to review the vast array of literature on pollinosis ("hay fever") and "vasomotor rhinitis".

The first paragraph is concerned with pollinosis and begins with a brief introductory note summarizing some of the earliest observations and experiments. Next, the aetiology and symptomatology of this condition are discussed and the influence of age and sex, family and personal history and the associated manifestations are described. Furthermore, the clinical course of pollinosis is outlined and the pathological changes of the nasal mucosa described. Finally, the characteristic laboratory findings and the different pictures of the nasal mucous membrane encountered on rhinoscopic examination, are pointed out.

In the second paragraph of this chapter "vasomotor rhinitis" is discussed. A review of the literature reveals a diversity of terminology for the non-seasonal confined-nasal symptoms. Different aetiological factors have been considered namely, allergy, non-specific hyperreactivity, bacteria, endocrine dysfunction, drugs and an unbalanced autonomic nervous system.

Further, the symptomatology has been discussed, the influence of sex and age, family and personal history, and the associated manifestations are described. The likely clinical course has also been sketched. Furthermore, the pathological changes occurring in the nasal tissues and the laboratory findings, are described and compared with those seen in pollinosis patients. Finally, the clinical pictures of the nasal mucous membrane encountered on rhinoscopic examination, are mentioned.

This chapter ends with a differential diagnosis of pollinosis, allergic and non-allergic rhinopathy.

The clinical differentiation from nasal viral affections was considered not relevant for this investigation and is therefore not discussed.

Chapter III

This chapter deals with the assessment of the reactivity of the nasal respiratory mucosa. In the introduction (§ 1) it is pointed out that the stimulus and its effects, the measurement of the effect, and the relationship between the stimulus and the effect, should be explicitly described in order to have a working notion of the "reactivity" of the nasal respiratory mucosa.

Histamine is most commonly used as the stimulus and its effects are briefly characterized.

For the measurement of the effect, it is stated that it should be measured directly, which however, requires direct intranasal manipulation. The anatomy of the nose makes it difficult to carry out these direct measurements. The disadvantages of these direct methods are further briefly outlined and it is concluded that changes in the nasal passage is the most appropriate parameter for the measurement of the reaction of the nasal mucosa, since changes in the nasal mucosa — either decongestion or congestion and probably hypersecretion, will cause changes in the nasal passage.

In the second paragraph, a brief review of the literature is given concerning the different methods applied by various investigators for the measurement of the nasal passage. It is concluded that the direct measurement of the pressure in the nasopharyngeal cavity is the method of choice and that therefore posterior rhinometry is preferable to anterior rhinometry.

The third paragraph deals with the author's method for the determination of the reaction pattern of the nasal respiratory mucosa to histamine.

The method consists of the simultaneous registration of the pharynx-nostril pressure gradient and tidal volume via one nasal chamber during normal respiration; by means of an additional device, changes in the oesophageal pharynx pressure gradient can also be obtained. The procedure and the mode of application of the stimulus (histamine) are related in detail. The problem of estimating the reactivity of the nasal respiratory mucosa from the relationship between the stimulus (histamine) and the effect (changes in the nasal passage) is discussed in § 4.

Different criteria are postulated, discussed and compared and finally it is concluded that the minimal x (exponent of 2) of 2^x mg/ml histamine, which causes a reaction of at least twice the initial pharynx-nostril pressure gradient (= "$2P_i$"), is the best criterion for the determination of the nasal revativity. This criterion has been applied in all further clinical and epidemiological investigations.

In the fifth paragraph, some possible sources of error in this assessment of the reactivity of the nasal respiratory mucosa, namely, the influence of mechanical irritation (repeated intranasal application) and of the solvent of histamine are discussed. From the results the conclusions are drawn that both these possible sources of error can be neglected.

The next paragraph (§ 6) is concerned with two questions: (1) whether a difference exists in the reactivity of the nasal mucosa when the mode of histamine application is changed (topical application or nebulization, and (2) whether bronchial reactions occur when the histamine is nebulized or topically applied. During topical application the reaction of the nasal mucosa occurs at lower concentrations in comparison to nebulization (mean difference: —2.2 concentration steps). This difference is thought to be due to a higher

concentration of histamine reaching the nasal mucosa with topical application.

In 3 patients (out of a group of 25) an increase of the oesophageal pressure gradient has been found following topical histamine application, although the bronchial histamine threshold values (determined beforehand by inhalation) were much higher than those of the nasal mucosa. It is argued that these changes are due to either, a reaction of the lower respiratory tract caused by local absorption of histamine, or due to a direct influence of the immediate increase of pharyngeal pressure and subsequent increase of the pressure in the lower respiratory tract on the calibre of the bronchi, or due to neurogenic factors from the nasal mucosa or nasopharynx.

Paragraph 7 is concerned with the influence of the anatomical structure on the nasal histamine reactivity. Pilot studies were done to answer 2 questions: (1) whether a relationship exists between the initial pharynx-nostril pressure gradient and the nasal histamine reactivity — and (a) whether a difference exists in the nasal histamine reactivity of the left and right nasal cavities in subjects with a clear - cut anatomical lesion, e.g. septum deviation.

In a random patient group with nasal complaints, the analysis of the results reveals no relationship between the initial pharynx-nostril pressure gradient and the nasal histamine reactivity, but when there is a clear-cut anatomical lesion (septum deviation) the histamine reactivty of nasal mucosa is found to be increased on the narrowed side. It is concluded that the nasal reactivity is, to a certain extent, influenced by the anatomical structure, but that the anatomical factor is of no major disturbing influence in the assessment of the nasal histamine reactivity. Differences in reaction are therefore probably due to a fundamental factor, rather than incidental causes.

In paragraph 8, a good reproducibility of the nasal histamine reactivty is demonstrated (table 22).

After a brief review of the literature concerning the so-called nasal cycle, the author's results are given. It has been found that in 5 normal subjects, without nasal complaints, an alternating cycle of reaction occurs in the nasal mucosa of both sides; in 5 patients with nasal complaints, this alternating cycle is disturbed. The nasal cycle therefore, probably does not play a disturbing rôle in the reproducibility of the nasal histamine reactivity.

The last paragraph of this chapter deals with complications encountered in the course of this investigation.

Chapter IV

In chapter IV the clinical significance of the nasal histamine reactivity is considered and some epidemiological studies are reported. It has been shown (§ 2) that a relationship exists between the nasal complaints and the histamine reactivity of the nasal mucosa. Furthermore, in this group of patients, an "age effect" on the nasal histamine reactivity is demonstrated, viz. a high degree of the nasal histamine reactivity in the younger age groups (10-40, but mainly 14-29 years) and a lower degree in the subjects over 40 years of age. In this regard, the necessity of an epidemiological study is stressed for a better judgement, since the results reported in § 2, were obtained from selected patients.

Next (§ 3), after an introductory note on epidemiological studies in the past and at present, a brief review of the literature is presented concerning the prevalence of nasal complaints. From these epidemiological reports it is concluded, that apparently no age effect is involved in the prevalence of nasal complaints in subjects over 40 years of age,* that a predominance of males exists, and that an "environmental factor" and a seasonal influence appear to be of importance. The effect of cigarette smoking has been mentioned as to be of no importance in the prevalence of nasal complaints.

In the same paragraph (§ 3), the results of the author's epidemiological surveys are presented in detail. These epidemiological surveys have been carried out in random samples of the populations (subjects over 40 years of age) of two communities, namely *Sellingen,* localized in an agricultural area free from industrial air pollution (1060 subjects investigated: 539 males and 521 females), and *Vlaardingen* (near Rotterdam), situated in an industrial polluted area (1188 subjects investigated: 651 males and 537 females).

A concise resumé of these results, given in detail in chapter IV, will be presented here:

1. A higher prevalence of nasal complaints is found in the random sample of Vlaardingen as compared with Sellingen, which probably means that "environmental factors" play a rôle in the prevalence of nasal complaints also in this country.

* No epidemiological report has been found on the prevalence of nasal complaints in subjects under 40 years of age.

2. No definite age effect is found on the prevalence of nasal complaints.
3. A predominance of males exists.
4. Nasal complaints (obstruction, sneezing and hypersecretion) are found to co-exist in a significant degree.
5. A positive relationship has been found between nasal and chest complaints.
6. A striking difference, in respect to the nasal histamine reactivity, has been found between the two random samples and our patient group, namely, a predominance of subjects in the patient group reacting at the lower histamine concentrations (table 34).
7. A marked difference concerning the nasal histamine reactivity between the two random samples was also present: more subjects from the sample of Vlaardingen with lower histamine threshold values. This finding has been interpreted as the result of the influence of an environmental factor (industrial air pollution).
8. A significant relationship exists between the nasal complaints and the histamine reactivity of the nasal respiratory mucosa in the two random samples.
9. A comparison of the two randam samples reveals a striking difference: the percentage of subjects without nasal complaints with a low degree of nasal histamine reactivity, viz. $\geqq 2^5$ mg/ml ($\geqq +5$), in the Sellingen group (49 %), is nearly three times higher than in the Vlaardingen group (18 %), whereas a predominance of individuals with nasal complaints and a nasal histamine reactivity of $\leqq 2^3$ mg/ml ($\leqq +3$), is present in the Vlaardingen sample. In cases with one single nasal symptom, the nasal histamine reactivity is increased mainly in those with only nasal obstruction or sneezing in contrast to those with nasal hypersecretion.
10. No age effect on the nasal histamine reactivity can be demonstrated (see fig. 11).
11. A predominance of males with lower histamine threshold values in comparison with the females is found; when the nasal complaints are taken into account no sex difference, however, is present.
12. A "normal" histamine threshold value has been suggested to be 8 mg/ml (see table 86).

Table 86

Prevalence of persons without nasal complaints with "normal" histamine reactivity of the nasal mucosa with different postulated normal values.

"Normal values" in mg/ml	% normal cases Sellingen	Vlaardingen
> 32	87	45
≥ 32	88	52
≥ 8	95	64
≥ 2	100	90
≥ 0.5	100	95
≥ 0.125	100	100

13. No statistically significant relationship is present between the initial pharynx-nostril pressure gradient and the nasal histamine reactivity, and no association has been demonstrated between the initial pharynx-nostril pressure gradient and the nasal complaints in the two random samples.
14. However, a comparison of the two random samples (with nasal complaints) in respect to the initial pharynx-nostril pressure gradient, reveals a slight but statistically significant difference. The same holds true for those without nasal complaints, although the difference between the mean values is not significant. We have not been able to explain the difference in the initial pharynx-nostril pressure gradient between the two random samples.

Chapter V

Chapter V deals with the effect of different pharmacological agents on the nasal respiratory mucosa in man. A brief introduction (§ 1) summarizes our knowledge to date concerning the mediation of allergic (specific) and non-allergic (non-specific) reactions in the human being. It is pointed out that this is a field which is still developing and as yet remains largely descriptive.

Attention has been paid to certain effects of these pharmacological agents, namely, the effect on:

— smooth muscle
— blood vessels
— capillary permeability
— mucous glandular secretion
— eosinophils.

Following the review of the literature concerning these effects in laboratory animals and in the human being, the author's experiments are given.

The pharmacological substances used in this study, are: acetylcholine, 5-hydroxytryptamine, bradykinin, a histamine releasing agent (compound 48/80), adrenaline and isoproterenol. The methods and materials are described, data regarding the patients are given, and the effects of these substances on the nasal respiratory mucosa in these patients are compared with those of histamine. The results can be summarized as follows:

1. Histamine causes a marked swelling of the nasal respiratory mucosa (sometimes accompanied by increased secretion and sneezing).
2. Acetylcholine causes an increased nasal secretion, sometimes accompanied by swelling of the nasal respiratory mucosa.
3. 5-Hydroxytryptamine (2 %), bradykinin (0.1 %) and compound 48/80 (1 mg/ml) have no effect on the nasal respiratory mucosa, as applied in this study.
4. Adrenaline causes a marked decongestion of the nasal respiratory mucosa, while the application of isoproterenol to the nasal respiratory mucosa results in a swelling.

Chapter VI

In the sixth chapter a description is given concerning the experimental investigation of the effects of allergens on the nasal respiratory mucosa and the bronchial tree, and their relationship with the histamine reactivity.

The first paragraph is a general introduction about the allergic reaction, and the allergens are classified according to their mode of entrance into the human body.

A concise resumé of the literature concerning the nasal and bronchial provocative tests from the earliest reports to date, is presented. Finally, the problems involved in the evaluation of the nasal and bronchial provocative tests are discussed.

The following questions are raised:

A. Does a relationship exist between the results of the nasal provocavite tests with allergens and the clinical condition [e.g. pre-

sence or absence of an actual pollinosis (actual nasal complaints), presence or absence of a history of actual pollinosis (nasal complaints), positive or negative skin tests]?

B. Does a relationship exist between the nasal reaction to allergens and to histamine?

C. Does a relationship exist between the reactivity of the nasal respiratory mucosa and the bronchial tree to allergens?

In the second paragraph of this chapter, the author's methods are reported in detail and the selection of the patients is outlined, while a full account of the results of the provocative tests with pollen, house dust, moulds and epidermals, is presented successively. These results can briefly be summarized as follows:

ad A. *Relationship between the results of the nasal provocative test and the clinical condition*

In the case of a positive history of *manifest* pollinosis, the nasal provocative test has been found to be highly specific, viz. in every subject with manifest pollinosis, a positive nasal test has been found; in all these subjects a positive intracutaneous test to pollen has also been found.

Manifest pollinosis has been found to be less frequent over 40 years of age; in those encountered, a reaction of the nasal mucosa usually occurs at higher pollen concentrations than in patients under 40 years of age.

In subjects with a history of pollinosis but without manifest pollinosis anymore (subjects over 40 years of age) no reaction of the nasal respiratory mucosa has been found following the provocation of pollen, although a positive intracutaneous test has been found in all these individuals. In subjects with a negative history of pollinosis, but with a positive intracutaneous test to pollen, no reaction of the nasal respiratory mucosa to pollen has been found.

On the other hand, in the case of house dust, moulds and epidermals, a negative nasal provocative test is present not only in subjects without nasal complaints, but also in those with nasal complaints; however, the nasal complaints cannot be attributed with

certainty to an allergen suspected by the intracutaneous test — but, it has been found that when the nasal provocative test is positive, the patient has also nasal complaints and a positive intracutaneous test to the allergen concerned. In general, in these cases, higher numbers of positive nasal provocative tests are found in subjects under 40 years of age as in the case of pollinosis.

ad B. *Relationship between the nasal histamine reactivity and the reaction of the nasal respiratory mucosa to allergens*

A significant relationship is present between the degree of the nasal histamine reactivity and the presence of a positive or negative provocative test with an allergen, viz. in the case of a positive provocative test, the reaction occurs at lower histamine concentrations.

ad C. *Relationship between the nasal and bronchial reactions to allergens*

Generally, a correlation has been demonstrated between the bronchial tree and the nasal respiratory mucosa with respect to positive and negative bronchial and nasal provocative tests.

All patients under 40 years of age with manifest pollinosis, reacted with the nasal respiratory mucosa *and* the bronchial tree to pollen provocation.

In subjects over 40 years of age with manifest pollinosis, positive nasal *and* bronchial pollen provocative tests have been found in 8 out of 10; 2 subjects with positive nasal reactions did not respond to the inhalation of pollen. In 11 patients (over 40 years of age) with a history of pollinosis but, without manifest pollinosis anymore, a negative nasal provocative test has been found in 9 subjects who did respond with bronchial obstruction to the inhalation of pollen; in the other 2 patients no reaction of the nasal respiratory mucosa or bronchial tree has been found. In the case of the other allergens, however, the nasal complaints cannot be explained with certainty to be caused by a certain allergen especially in those with negative nasal provocative tests (see table 87).

Table 87

The relationship between the nasal and the bronchial provocative tests in subjects under and over 40 years of age with nasal and chest complaints, and with positive intracutaneous tests to house dust, moulds or epidermals.

Allergens		≤ 40 years of age AL_B		> 40 years of age AL_B	
		+	—	+	—
House dust	AL_N +	27	11	4	3
	AL_N —	6	23	7	9
Moulds	AL_N +	9	6	2	0
	AL_N —	1	9	3	8
Epidermals	AL_N +	5	4	1	2
	AL_N —	1	13	2	5

AL_N: Nasal provocative test, positive (+) or negative (—).
AL_B: Bronchial provocative test, positive (+) or negative (—).

In the last paragraph (§ 4) of this chapter, the relationship between the degree of the histamine reactivity of the nasal respiratory mucosa and the bronchial tree, is discussed.

From the results, a statistically significant relationship between the degree of the nasal and the bronchial histamine reactivity has been found only in subjects under 40 years of age with positive nasal and bronchial provocative tests to allergens [these patients are further characterized by the presence of nasal complaints (or manifest pollinosis), CNSLD, and positive intracutaneous tests]. In cases without CNSLD and nasal complaints (limited numbers) all reactions are negative.

Chapter VII

Chapter VII gives a representation of the author's experiments with non-specific (non-allergic) stimuli on the nasal respiratory mucosa.

In the introduction (§ 1) non-specific (non-allergic) reactions, occurring in the nasal mucosa, are defined.

The second paragraph is concerned with the experiments with sulphur dioxide (SO_2) just as a parameter for aspecific stimulation on the nasal respiratory mucosa. The method of assessment of the nasal reaction is mentioned and the patients (total: 10) are charac-

terized. The reaction of the nasal mucosa to SO_2 is compared with that to histamine. The results reveal that no reaction of the nasal mucosa occurs on topical application of SO_2, while histamine in these cases, has a pronounced effect. In the same paragraph, another trial with SO_2 is described, i.e. the simultaneous assessment via one nasal chamber of the reaction of the nasal mucosa and the bronchial tree to nebulized SO_2, in patients (total: 10) with nasal and chest complaints. All these patients had an increased histamine reactivity (low threshold values) of the nasal mucosa and of the bronchial tree.

Severe bronchial obstruction occurred in 8 of the 10 patients following nebulization of SO_2. In 5 of the 10 patients, increased nasal secretion was noted, while 2 of them also showed a slight swelling of the nasal mucosa.

In the third paragraph, a full account is given of the experiments with topical application of ammonia solutions on the nasal mucosa in 20 patients with nasal and chest complaints. The nasal reaction to ammonia has also been compared with that to histamine.

From the results, a relationship has been found between the degree of the nasal histamine reactivity and the nasal reaction to ammonia, but the comparison of the nasal reactions to histamine and ammonia reveals a different pattern, i.e. following histamine application, swelling of the nasal mucosa is predominant, whereas in the case of ammonia provocation, nasal hypersecretion is most conspicuous.

Final conclusions

1. Various factors apparently influence the reaction of the nasal respiratory mucosa and the bronchial tree. Therefore, it is unknown in how far the results given and conclusions drawn, can be generalized.
2. The reaction of the nasal respiratory mucosa to histamine (and to other stimuli) can be assessed by measuring changes in the nasal passage. The parameter applied seems suitable.
3. A significant relationship exists between nasal complaints and the histamine reactivity of the nasal respiratory mucosa as was demonstrated in patients and in two random samples of "normal" populations: a lower degree of reactivity was found

in normal populations than in persons with nasal complaints.

4. A significant relationship exists between nasal and chest complaints.
5. An age effect exists on the nasal histamnie reactivity, viz. a high degree of the nasal histamine reactivity in the younger age groups (10-40 years) and a lower degree in subjects over 40 years of age.
6. The nasal complaints in the case of a positive history of *manifest* pollinosis, can be attributed to pollen (allergy); however, in the case of other allergens, the nasal complaints cannot be attributed with certainty to a certain allergen only.
7. Manifest allergy has been found to be less frequent over 40 years of age.
8. No positive relationship has been found between the nasal- and the bronchial reactivity to histamine.
9. The reaction of the nasal respiratory mucosa and the bronchial tree to allergens, runs parallel in subjects under 40 years of age. This similar pattern does not necessarily mean that the (patho)physiological processes are identical.
10. A significant relationship exists between the reaction of the nasal respiratory mucosa to histamine and to allergens, viz. in the case of a positive provocative test, the reaction occurs at lower histamine concentrations in contrast to negative provocative tests. As in the case of pollinosis, this reactivity has not been measured in the pollen season, it is therefore suggested to consider this phenomenon as the cause of the allergic reaction; however, the epidemiological data also suggest other factors.
11. No positive relationship exists between the bronchial reaction to histamine and that to allergens.
12. The following factors should be considered in the interpretation of the degree of the histamine reactivity of the nasal respiratory mucosa:
    - mechanical factors
    - exogenous factors
    - age effect
    - the number of smooth muscle elements in different parts of the vascular system
    - an increased susceptibility of the separate contractile elements

APPENDIX I

Serial No. . . . . .

NAME: . . . . . .
ADDRESS: . . . . . .
DATE OF BIRTH: . . . . . .
SEX: . . . . . .
AGE: . . . . . .
DATE OF EXAMINATION: . . . . . .

A. **Nasal obstruction**

	Yes	No
Are you ever troubled by nasal obstruction?	. . . .	. . . .
If yes, when?		
During the day	. . . .	. . . .
In the evening	. . . .	. . . .
At night	. . . .	. . . .
In the morning	. . . .	. . . .
Frequency Every day	. . . .	. . . .
Every week	. . . .	. . . .
Every month	. . . .	. . . .
Once or a few times a year	. . . .	. . . .

B. **Hypersecretion**

Are you ever troubled by increased nasal secretion?	. . . .	. . . .
If yes, is it attended or not with nasal obstruction?	. . . .	. . . .
During the day	. . . .	. . . .
In the evening	. . . .	. . . .
At night	. . . .	. . . .
In the morning	. . . .	. . . .
Frequency Every day	. . . .	. . . .
Every week	. . . .	. . . .
Every month	. . . .	. . . .
Once or a few times a year	. . . .	. . . .
Colour of secretion watery	. . . .	. . . .
yellow	. . . .	. . . .
bloody	. . . .	. . . .

C. **Sneezing**

	Yes	No
Are you ever troubled by sneezing attacks?	. . . .	. . . .
If yes, are they usually attended with nasal obstruction and/or increased secretion?	. . . .	. . . .
When are the sneezing attacks?		
During the day	. . . .	. . . .
In the evening	. . . .	. . . .
At night	. . . .	. . . .
In the morning	. . . .	. . . .
Frequency Every day	. . . .	. . . .
Every week	. . . .	. . . .
Every month	. . . .	. . . .
Once or a few times a year	. . . .	. . . .

D. **Lacrimation:**

Are you troubled by lacrimation (watery or "tearing eyes")?	. . . .	. . . .
If yes, is it usually attended by sneezing?	. . . .	. . . .
nasal obstruction?	. . . .	. . . .
increased secretion?	. . . .	. . . .
tickeling or itching at the back of your throat?	. . . .	. . . .

E. **Headache**
Attended with nasal obstruction? ....
Localization: above the eyes? ....

F. **Influence of season**
Nasal obstruction (A) ....
Hypersecretion (B) ....
Sneezing (C) ....
January ....
February ....
March ....
April ....
May ....
June ....
July ....
August ....
September ....
October ....
November ....
December ....

G. **Allergy - Hyperreactivity**

	Yes	No	Specification of complaints
House dust	....	....	.....
Hay dust	....	....	.....
Moulds	....	....	.....
Pollen	....	....	.....
Flour products	....	....	.....
Epidermal products (cat, dog, horse, chicken, etc.)	....	....	.....
Specification (e.g. eidermal products or food)			

Effect of weather
Does the weather affect your nose? ....
If yes,
change from cold to warm ....
change from warm to cold ....
fog ....
rain ....
freeze cold ....
chemical or other irritants ....
(Nasal obstruction = A ....
Hypersecretion = B ....
Sneezing = C) ....

I. Are you troubled by:
Smoke (polluted air) ....
Tobacco smoke ....
Smelling of baking or frying ....

APPENDIX II

QUESTIONNAIRE ON NASAL SYMPTOMS

Enquete No. .....
Serial No. .....

NAME: .....
ADDRESS: .....

Age .....

M F

Sex ....

	day	month	year
Date of birth	....	.....	..
Date of interview	....	.....	..

1. **Nasal obstruction**

	Yes	No
Are you ever troubled by nasal obstruction?	....	....
Seldom	....	....
Periodically	....	....
Most days of the year	....	....

2. **Hypersecretion**

	Yes	No
Are you troubled by increased nasal secretion?		
Seldom	....	....
Periodically	....	....
Most days of the year	....	....

Colour of secretion:

watery	....
yellow	....
bloody	....

3. **Sneezing attacks**

	Yes	No
Are you ever troubled by sneezing attacks?	....	....
Seldom	....	....
Periodically	....	....
Most days of the year	....	....

APPENDIX III

QUESTIONNAIRE ON RESPIRATORY SYMPTOMS

Serial No. .....

NAME: .....
ADDRESS: .....

Age .....

M F

Sex ....

	day	month	year
Date of birth	....	.....	..
Date of interview	....	.....	..

A. **History**

Use actual wording of each question

Cough

		Yes	No
1.	Do you usually cough on getting up in the morning? (Count a cough with first smoke or on "first going out of doors". Exclude clearing throat or a single cough).	....	....
2.	Do you usually cough during the day or at night?	....	....
3.	Do you cough like this on most days for as much as three months per year?	....	....
4.	For how many years have you coughed like this? 2 numbers .... year		

Sputum

5.	Do you usually bring up any phlegm from your chest on getting up in the morning? (Count phlegm with the first smoke or on "first going out of doors". Exclude secretion from nose).	....	....
6.	Do you usually bring up phlegm from your chest during the day or at night? (Accept twice or more).	....	....
7.	Do you bring up phlegm like this on most days for as much as three months per year? If „yes",	....	....
8.	For how many years have you brought up phlegm? 2 numbers .... year		
9.	What is the colour of the phlegm: green or yellow?	....	
10.	In the past three years, have you had a period of (increased)* cough and phlegm lasting for 3 weeks or more? one period .... two or more periods .... doubt		.... no

Breathlessness

		Yes	No
11.	Are you troubled by shortness of breath? (If disabled from walking by any condition other than heart or lung disease put "X" here)	....	....
12.	Are you ever troubled by shortness of breath, when hurrying on the level or walking up a slight hill? (If "no", grade 0. If "yes", proceed to next question).	....	....
13.	Do you get short of breath walking with other people at an ordinary pace on the level? (If "no", grade 1. If "yes", proceed to next question).	....	....
14.	Do you have to stop for breath when walking at your own pace on the level? (If "no", grade 2. If "yes", proceed to next question).	....	....
15.	Are you short of breath on washing or dressing? (If "no", grade 3. If "yes", proceed to next question).	....	....
16.	Do you already get short of breath at rest? (If "no", grade 4. If "yes", proceed to next question).	....	....
17.	At what age did you notice that you were getting short of breath? (Accept "yes" if answered "about this time"). 2 figures .... year		

* For subjects who cough and bring up phlegm.

Wheezing

18. Does your chest ever sound wheezy or whistling? ....
19. Do you get this most days or nights? ....

Paroxysmal attacks of dyspnoea

20. Have you ever had attacks of dyspnoea (asthmatic attacks) accompanied by wheezing from your chest? ....
21. At what age did these attacks start? 2 figures .... year
22. Are you more troubled at present by your chest than one month ago? ....

Non-tuberculosis affections of the respiratory tract

23. During the past 3 years have you had any chest illness which has kept you off work, indoors, at home or in bed? ....
(If "yes", ask details of each illness).

Year	Duration of affection: Less than one week	Duration of affection: One week or more	Increased phlegm: Yes	Increased phlegm: No	Doctor's diagnosis

B. **Physical Examination**

	Yes	No
a. Wheezing or râles	....	....
b. Moist rhonchi	....	....
c. Prolonged expiration	....	....

APPENDIX IV

THE INTRACUTANEOUS TEST

Technique

For this test, a sterile syringe with 0.1 graduations, and fitted with a half-inch (13 mm) intradermal needle is used.

The skin sites (inneraspect of patient's forearm) are cleansed superficially with cotton moistened with ether. The needle is inserted (with the bevel up and almost parallel with the surface of the skin) into the epidermis obviated by introducing the bevel of the needle far enough to pick the skin up, and then injecting the liquid extract into the skin and not beneath it. The amount of solution (0.04-0.05 cc) is used consistent throughout. Following the injection of the sterile liquid allergen (dilute extracts employed for routine testing: (grass) pollen 1000 N.U., house dust 0.5 mg/ml, moulds 0.2 mg/ml, epidermals 0.25 mg/ml) a small whitish pin-point elevation should be visible - ± 4 mm in diameter. Coca's solution (employed for diluting the extracts) is used for the control injection.

The sites for testing should be at least 1 inch (2.5 cm) apart laterally and 1 inch apart longitudinally. Ten minutes after tests have been applied, the reactions are evaluated and recorded. Before intracutaneous testing, the scratch test is performed.

Reading and recording of reactions

Each reaction, including that of the control injection, is interpreted and recorded in terms of the diameter of the wheal, using "plus" or "minus" symbols to represent the various diameters. The reactions are read as follows:

(a) — : Here, there is no increase in the size of the original papule and resembles that of the control.
(b) + : An increase in size of wheal not more than 7.5 mm.
(c) +± : Wheal between 7.5 and 10 mm.
(d) ++ : Wheal between 10 and 12.5 mm.
(e) +++ : Wheal between 12.5 and 15 mm.
(f) ++++ : An increase in size of wheal more than 15 mm, sometimes with irregular projections ("pseudopodia") and a considerable area of surrounding erythema.

APPENDIX V

ALLERGENS TESTED

Control solution (Coca)
Histamine 0.01 mg/ml
House dust 0.5 mg/ml
Moulds: A 0.2 mg/ml
B 0.2 mg/ml
C 0.2 mg/ml
D 0.2 mg/ml
E 0.2 mg/ml
Grass pollen 1000 Noon Units
Epidermals 0.25 mg/ml
Specification of the combined extracts:

I.

Moulds A:
1 Trichoderma viride
2 Fusarium culmosum
3 Cladosporium oides
4 Cladosporium elatum
5 Cladosporium herbarum
6 Rhizopus nigricans
7 Stemphylium botryosum
8 Altenaria tenuis
9 Pen. brevi compactum
10 Pen. expansum
11 Pen. notatum
12 Pen. frequentans
13 Pen. commune
14 Aspergillus versicolor
15 Aspergillus niger
16 Aspergillus fumigatus
17 Mucor spinosus
18 Mudor mucedo
19 Mudor racemosus
20 Pullularia pullulans
21 Botrytis cenera
22 Merculius domesticus
23 Epicoccum purpurascens

Moulds B:
1 Aspergillus candidus
2 Aspergillus ochraceus
3 Aspergillus terreus
4 Aspergillus restriculus
5 Aspergillus clavalus
6 Aspergillus arustelodemi
7 Aspergillus nidulans
8 Aspergillus flavus

Moulds C:
1 Absidia ramosa
2 Aleurisma carmis
3 Scopulariopsis brevicaulis
4 Papularia sphaerosperma
5 Fusarium solani
6 Penicillium digitatum
7 Oidiodendras griseum

Moulds D:
1 Eidarnella spinosa
2 Pestutitia populini
3 Geotrichem candidum
4 Iritirachum dependens
5 Stysanas stemonitis
6 Neurospara sitophila
7 Trichothecium roseum
8 Chactonium funicola

Moulds E:

1 Amblyosporium botrytis
2 Ascochyta pisi
3 Arachniotus trisporus
4 Bispora effusa
5 Botrytis crystallina
6 Calderriomyces fumago
7 Cephalosporium lamellaecola
8 Cephalosporium stühmeri
9 Chloridium minus
10 Circinella minor
11 Clasterosporium carpophilum
12 Curvularia lunata

II. Grass pollen:

1 Secale cereale
2 Dactylis glomerata
3 Lolium perenne
4 Anthoxantum odoratum
5 Alopecurus pratensis
6 Agrostis alba
7 Holcus lanatus
8 Cynosurus christatus

III. Epidermals:

1 Horse
2 Pig
3 Cat
4 Goat
5 Cattle
6 Rabbit
7 Dog
8 Sheep
9 Man
10 Feathers

Abel, J. J., Crawford, A. C. (1897) Bull Johns Hopk. Hosp. **8,** 151.
Abel, J. J., Crawford, A. C. (1899) Z. phys. Chem. **28,** 318.
Adam, H. M., Hunter, R. B., Kinnear, T. W. G. (1951) Quart. J. exp. Physiol. **36,** 49.
Adam, H. M., Hardwich, D. C., Spencer, K. E. V. (1957) Brit. J. Pharmacol. **12,** 397.
Adam, J., (1900) Glasg. hosp. Rep. **3,** 151.
Adami, M. (1930) J. G. Adami: A Memoir. (Constable, London).
Ado, A. D., Adrianova, N. W., Acountz, V. B., Povochina, Y. A. (1965) In: Abstracts of Symposia. The Sixth European Congress of Allergology, Stockholm. p. 33.
Agner, K. (1950) J. exp. Med. **92,** 337.
Ahlquist, R. P. (1948) Amer. J. Physiol. **153,** 586.
Ahlquist, R. P. (1962) Arch. int. Pharmacodyn. **139,** 38.
Aird, R. B. (1959) Ann. Allergy **17,** 905.
Alam, M., Anrep, G. V., Barsoum, G. S., Talaat, M., Wieninger, E. (1939) J. Physiol. **95,** 148.
Albert, M. M. (1960) In: Fundamentals of Modern Allergy. Ed. Prigal, J. (Mc Graw-Hill Book Co., Inc., New York) p. 189.
Alcock, P., Berry, J. L., Daly de Burgh, I. (1935) Quart. J. exp. Physiol. **25,** 369.
Aldrete, J. S., Sheps, S. G., Bernatz, P. E., Didier, E. P. (1966) Mayo Clin. Proc. **41,** 399.
Aldrich, T. B. (1905) J. amer. chem. Soc. **27,** 1074.
Alexander, R. S. (1963) In: Handbook of Physiology Section 2: Circulation II. Ed. Hamilton, W. F., Dow, P. (Williams & Wilkins, Baltimore) p. 1075.
Alksne, J. F. (1959) Quart. J. exp. Physiol. **44,** 51.
Allwood, M. J., Lewis, G. P. (1963) J. Physiol. **166,** 47.
Allwood, M. J., Lewis, G. P. (1964) J. Physiol. **170,** 571.
Alyea, O. E. van (1955) Ann. Otol. Rhinol. Laryng. **64,** 192.
Ambache, N., Barsoum, G. S. (1939) J. Physiol. **96,** 139.
Anderson, J. R. (1954) Ann. Allergy **12,** 627.
Anderson, J. R. (1956) Ann. Allergy **14,** 44.
Anrep, G. V., Ayadi, M. S., Barsoum, G. S., Smith, J. R., Talaat, M. (1944) J. Physiol. **103,** 155.
Arbeiter, H. I. (1961) Ann. Allergy **19,** 633.
Arbesman, C. E., Girard, P., Rose, N. R. (1964) J. Allergy **35,** 535.
Archer, R. K. (1959) J. Path. Bact. **78,** 95.
Archer, R. K. (1960) Nature **187,** 155.
Archer, R. K., Feldberg, W., Kovacs, B. A. (1962) Brit. J. Pharmacol. **18,** 101.
Archer, R. K., Broome, J. (1963) Nature **198,** 893.
Archer, R. K., Broome, J. (1963) Acta haemat. **29,** 147.
Archer, R. K. (1963) The Eosinophil Leucocytes (Blackwell, Oxford).
Ariëns, E. J., Rossum, J. M. van, Simonis, A. M. (1957) Pharmacol. Rev. **9,** 218.
Ariëns, E. J., Rossum, J. M. van (1957) Arch. int. Pharmacodyn. **110,** 275.
Ariëns, E. J. (1960) In: Adrenergic Mechanisms. Ciba Foundation Symposium. Ed. Vane, J. R., Wolstenholme, G. E. W., O'Connor, M. (Churchill, London) p. 253 & 264.
Ariëns, E. J., Simonis, A. M. (1960) Arch. int. Pharmacodyn. **172,** 479.
Ariëns, E. J., Simonis, A. M. (1962) Acta physiol. pharmacol. neerl. **11,** 151.
Ariëns, E. J. (1963) Molecular Pharmacology. (Academic Press, New York).
Ariëns, E. J. (1964) In: Gebruik en Misbruik van Neusdruppels en -Spray. Mededelingen Uitgegeven onder Auspiciën van de Stichting Experimenteel Onderzoek Allergie. (Ver. Grafische Bedrijven, Haarlem) p. 20.
Ariëns, E. J. (1964) In: Bronchitis, Second International Symposium. Ed. Orie, N. G. M., Sluiter, H. J. (Van Gorcum, Assen) p. 209.

Armitage, P., Herxheimer, H., Rosa, L. (1952) Brit. J. Pharmacol. **7,** 625.
Armstrong, D., Keele, C. A. (1954) Nature **174,** 791.
Armstrong, D., Jepson, J. B., Keele, C. A., Stewart, J. W. (1957) J. Physiol. **135,** 350.
Arner, B. (1948) Acta med. scand. Suppl. **239,** 327.
Arnoldsson, H., Helander, C. G., Helander, E., Lindell, S. E., Lindholm, B., Olsson, O., Roos, B. E., Svanborg, A., Söderholm, B., Westling, H. (1962) Scand. J. clin. Lab. Invest. **14,** 241.
Arsdel, P. P. van, Middleton, E., Sherman, W. B., Buchwald, H. (1958) J. Allergy **29,** 429.
Arsdel, P. P. van, Beall, G. N. (1960) Arch. intern. Med. **106,** 714.
Arunlakshana, O. (1953) J. Physiol. **119,** 47P.
Aschan, G., Drettner, B. (1957) Acta oto-laryng., Stockh. Suppl. 140.
Aschan, G., Drettner, B., Ronge, H. (1958) Ann. acad. Reg. Sci. Upsal. **2,** 111.
Attinger, E. O. (1957) Amer. Heart. J. **54,** 837.
Attinger, E. O. (1959) J. Pharmacol., Belg. **14,** 22.
Auerbach, O., Brewster, G. J., Forman, J. B., Petnick, T. G., Smolin, M. J., Muehsam, G. E., Kassoung, D. Y., Stout, A. P. (1957) New Eng. J. Med. **256,** 97.
Aviado, D. M., Schmidt, C. F. (1957) J. Pharmacol. exp. Ther. **120,** 512.
Aviado, D. M., Ling, J. S., Schmidt, C. F. (1957) Amer. J. Physiol. **189,** 253.
Aviado, D. M. (1960) Pharmacol. Rev. **12,** 159.
Aviado, D. M. (1960) Amer. J. Physiol. **198,** 1032.
Aviado, D. M. (1965) The lung Circulation, Volume I & II (Pergamon Press, Oxford).
Axelrod, J. (1959) Physiol. Rev. **39,** 751.
Bablik, L. (1965) Arch. Ohr.-Nas.-KehlkHeilk. **184,** 384.
Baeyer, A. (1867) Liebig's Annalen **142,** 322.
Balchum, O. J., Felton, J. S., Jamison, J. N., Gaines, R. S., Clarke, D. R., Owan, T., Industrial Health Committee and the Tuberculosis and Health Association of Los Angeles (1962) Amer. Rev. resp. Dis. **86,** 675.
Baldrighi, V., Ferrari, V. (1955) Folia cardiol. **14,** 7.
Baldwin, J. L. (1952) J. Lab. clin. Med. **39,** 737.
Ballenger, H. C. (1944) A Manual of Otology, Rhinology and Laryngology (Lea & Febiger, Philadelphia).
Baltzly, R., Buck, J. S., Beer, E. J. de, Webb, F. A. (1949) J. amer. chem. Soc. **71,** 1301.
Balyeat, R. M. (1929) Sth. med. J. **22,** 492.
Bang, F. B. (1961) Bact. Rev. **25,** 228.
Baravelli, P., Ceroni, T. (1962) Ther. Umsch. **19,** 97.
Barger, G., Dale, H. H. (1910) J. Physiol. **40,** 38.
Barger, G., Dale, H. H. (1910) J. Physiol. **41,** 19.
Bargmann, W. (1964) Histologie und mikroskopische Anatomie des Menschen (G. Thieme Verlag, Stuttgart).
Barsoum, G. S., Gaddum, J. H. (1935) J. Physiol. **85,** 1.
Barsoum, G. S., Gaddum, J. H. (1935) J. Physiol. **85,** 13P.
Bass, D. E., Henschell, A. (1956) Physiol. Rev. **36,** 128.
Batson, O. V. (1935) Ann. Otol. Rhinol. Laryng. **44,** 939.
Batson, O. V. (1954) Ann. Otol. Rhinol. Laryng. **63,** 571.
Baum, H. L. (1934) Arch. Otolaryng. **20,** 804.
Baum, H. L. (1935) Ann. Otol. Rhinol. Laryng. **44,** 500.
Bawden, H. (1901) J. comp. Neurol. **11,** 1.
Baxter, J. D., Rose, B. (1953) J. Allergy **24,** 18.
Beakey, J. F., Bresnick, E., Levinson, L., Segal, M. S. (1949) Ann. Allergy **7,** 113.
Beard, G. M. (1876) Hay Fever or Summer Catarrh. (New York).
Beckman, H. (1961) Pharmacology, the Nature, Action and Use of Drugs (Saunders, Philadelphia).

Behnke, R. H., Williams, J. F., White, D. H. (1963) Amer. Rev. resp. Dis. **87,** 57.
Beickert, P. (1950) Z. Hals-Nas.-Ohrenheilk. **157,** 136.
Beickert, P. (1951) Z. Hals-Nas.-Ohrenheilk. **157,** 404.
Bell, G. H., Davidson, J. N., Scarborough, H. (1965) Textbook of Physiology and Biochemistry (E. & S. Livingstone, Edinburgh).
Benditt, E. P., Rowley, D. (1956) J. exp. Med. **103,** 399.
Benditt, E. P., Arase, M., Roeper, E. (1956) J. Histochem. Cytochem. **4,** 419.
Benjamins, C. E. (1931) Ned. T. Geneesk. **75,** 2002.
Benjamins, C. E. (1932) Ned. T. Geneesk. **76,** 539.
Benjamins, C. E. (1932) Geneesk. T. Ned.-Indië **72,** No. 16.
Benjamins, C. E. (1932) Maandschr. Kindergeneesk. **II,** 49.
Benjamins, C. E. (1934) Ned. T. Geneesk. **78,** 2266.
Benjamins, C. E. (1935) Proceedings (Kon. Akad. Wetensch., Amsterdam) **38,** 308.
Benjamins, C. E., Dishoeck, H. A. E. van, German, J. L. M. (1935) Acta oto-laryng., Stockh. **24,** 153.
Benjamins, C. E., Dishoeck, H. A. E. van, German, J. L. (1935) J. Allergy **6,** 4.
Berdal, P. (1952) J. Allergy **23,** 11.
Berdal, P. (1954) Acta oto-laryng., Stockh. Suppl. 115.
Berendes, J., Link, R., Zöllner, F. (1964) Hals-Nasen-Ohren-Heilkunde, Band I (G. Thieme Verlag, Stuttgart).
Berglund, E. (1963) In: Mode of Action of Drugs. First International Pharmacological Meeting, 1961, Vol. 9, Part 2. Ed. Uvnäs, B. (Pergamon Press, Oxford) p. 101.
Berne, R. M. (1958) Circ. Res. **6,** 644.
Berry, P. A., Collier, H. O. J., Holgate, J. A. (1963) J. Physiol. **165,** 41P.
Best, W .R., Kark, R., Muehrcke, R., Samter, M. (1953) J. amer. med. Ass. **151,** 702.
Best, W. R. (1956) J. amer. med. Ass. **160,** 586.
Beumer, H. B. (1965) In: Abstracts. Sixth European Congress of Allergology, Stockholm (Ohlssons Boktryckeri, Lund) p. 35.
Beyne, J. (1926) Médicine (Sept.), p. 919.
Beyer, K. H. (1946) Physiol. Rev. **26,** 169.
Bhattacharya, B. K., Lewis, G. P. (1956) Brit. J. Pharmacol. **11,** 202.
Bhoola, K. D., Schachter, M. (1959) J. Physiol. **149,** 80P.
Bhoola, K. D., Collier, H. ●. J., Schachter, M., Shorley, P. G. (1962) Brit. J. Pharmacol. **19,** 190.
Biggart, J. H. (1932) J. Path. Bact. **35,** 799.
BIJL, W. J. F. van der (1961) In: Algemene Farmacologie. Ed. Lammers, W., Nelemans, F. A., Siderius, P. (Stafleu, Leiden) p. 656.
Billard, G. (1910) Gaz. hôp. **83,** 899.
Billiet, L., Baert, A., Woestijne, K. P. van de, Jamer, J., Gyselen, A. (1959) In: Inhalatietests en Inhalatie Therapie — Verslag van een Vergadering van de Begische en Nederlandse Verenigingen voor Allergie (H. E. Stenfert Kroese, Leiden) p. 47.
Binet, L., Burstein, M. (1946) J. Physiol. **39,** 7.
Binningerus, J. N. (1673) Observationum et Curationum Medicinalium, Cent. Quinque. Montbelgardi, Obs. **86,** 227.
Bishop, J. M., Harris, P., Bateman, M., Raine, J. M. (1962) Clin. Sci. **22,** 53.
Bishop, J. M., Harris, P., Segel, N. (1963) J. Physiol. **165,** 37P.
Bissett, G. W., Lewis, G. P. (1962) Brit. J. Pharmacol. **19,** 168.
Blackley, C. H. (1873) Experimental Researches on the Causes and Nature of Catarrhus Aestivus (Hay Fever). (London).
Blackley, C. H. (1880) Causes, Treatment and Effective Prevention (Bailliére, Tindall & Cox, London) p. 56.
Blier, X. (1930) Amer. J. Physiol. **93,** 398.
Bloch, W., Pinösch, H. (1936) Z. phys. Chem. **239,** 236.

Bloom, G. D. (1965) In: The Inflammatory Process. Ed. Zweifach, B. W., Grant, L., McCluskey, R. T. (Academic Press, New York) p. 355.
Blumstein, G. I. (1937) J. Allergy **8,** 321.
Blumstein, G. I. (1945) Ann. Allergy **3,** 341.
Bock, K. D., Dengler, H., Kuhn, H. M., Matthes, K. (1957) Arch. exp. Path. Pharmakol. **230,** 257.
Bodi, T., Siegler, P. E., Brown, E. B., Gershenfeld, M. A., Nodine, J. H. (1961) Ann. Allergy **19,** 386.
Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S. (1956) Pharmacol. exp. Ther. **117,** 82.
Boissonnas, R. A., Guttman, S., Jaquenoud, P. A., Konzett, H., Sturmer, E. (1960) Experientia **16,** 326.
Bolt, W. (1964) Bibl. anat. **7,** 92.
Boreus, L. O. (1960) Acta physiol. scand. **48,** 431.
Boreus, L. O. (1960) Acta physiol. scand. **50,** 375.
Borst, H. G., Berglund, E., McGregor, M. (1957) J. clin. Invest. **36,** 669.
Bostock, J. (1819) Med. chirurg. Trans., London **10,** 161.
Bostock, J. (1828) Med. chirurg. Trans., London **14,** 437.
Botallus, L. (1565) Commentariole duo Lugduni, p. 22 & 23.
Bouhuys, A., Jönsson, R., Lichtneckert, S., Lindell, S. E., Lundgren, C., Lundin, G., Ringquist, T. R. (1960) Clin. Sci. **19,** 79.
Bouhuys, A. (1963) Arch. environm. Hlth. **6,** 742.
Bouhuys, A. (1963) Amer. J. Med. **34,** 470.
Bourdillon, R. B., Lidmell, O. M. (1941) Lancet **2,** 365.
Boyland, E., Gaddum, J. H., McDonald, F. F. (1947) J. Hyg. **45,** 290.
Braun, K., Stern, S. (1961) Amer. J. Physiol. **201,** 369.
Braus, H. (1960) Anatomie des Menschen (Zweite Auflage Von Curt Elze) (Springer-Verlag, Berlin).
Bray, G. W. (1930) Brit. med. J. **1,** 384.
Bray, G. W. (1931) Recent Advances in Allergy (Churchill, London).
Bray, G. W. (1931) J. Allergy **2,** 205.
Bray, G. W. (1932) J. Allergy, **3,** 367.
Bray, G. W. (1937) Recent Advances in Allergy (Churchill, London).
Bresgen, E. (1881) Dtsch. med. Wschr. **34,** 595.
Brinkman, G. L., Coates, E. O. (1962) Amer. Rev. resp. Dis., **86,** 47.
Brocklehurst, W. E. (1953) J. Physiol. **120,** 16.
Brocklehurst, W. E. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 172.
Brocklehurst, W. E. (1960) J. Physiol. **151,** 416.
Brocklehurst, W. E. (1962) In: Progress in Allergy. Vol. 6. Ed. Kallós, P., Waksman, B. H. (Karger, Basel) p. 539.
Broder, I., Barlow, P. P., Horton, R. J. M. (1962) J. Allegy **33,** 513.
Brodie, B. B. (1956) J. Pharm. Pharmacol. **8,** 1.
Brodie, B. B., Shore, P. A. (1956/57) Ann. N. Y. Acad. Sci. **66,** 631.
Brodie, B. B. (1958) Amer. Rev. Biochem. **27,** 427.
Brodie, B. B., Costa, E. (1961) In: Hypertension, Recent Advances. Ed. Brest, A. N., Moyer, J. A. (Lea & Febiger, Philadelphia).
Brodie, T. G., Russell, A. E. (1900) J. Physiol. **26,** 92.
Brody, T. M. (1953) Pharmacol. Rev. **7,** 335.
Broom, J., Archer, R. K. (1962) Nature **193,** 446.
Brown, E. A. (1955) Quart. Rev. Allergy **7,** 51.
Brown, E. A. (1962) Ann. Allergy **20,** 397.
Brown, E. A. (1962) Ann. Allergy **20,** 744.
Brown, G. T. (1927) J. Lab. clin. Med. **12,** 1145.
Brown, J. H., Cook, K. M., Ney, F. G., Hatch, T. (1950) Amer. J. publ. Hlth **40,** 450.
Brown, W. B., Graham, I., Niedringhaus, A., Locke, A. (1943) J. Immunol. **46,** 101.

Brown, W. B., Mahoney, F., Niedringhaus, A., Locke, A. (1945) J. Immunol. **50,** 161.
Brubaker, A. P. (1919) J. amer. med. Ass. **73,** 585.
Bruce, R. A., Archer, R. K. (1962) Lancet **2,** 1119.
Bruce, R. A. (1963) Int. Arch. Allergy **22,** 294.
Bruck, F. (1901) Ther. Gegenw. **42,** 407.
Bruun, E. (1948) Acta med. scand. Suppl. **239,** 332.
Bruun, E., Letman, H. (1962) Acta allerg. **17,** 343.
Buchanan, A. R. (1962) Functional Neuro-Anatomy (Lea & Febiger, Philadelphia).
Buck, C. (1956) Amer. J. Hyg. **63,** 1.
Buckland, F. E., Tyrrell, D. A. J. (1964) J. Hyg. **62,** 365.
Buhrmester, C. C. (1936) Ann. Otol. Rhinol. Laryng. **45,** 687.
Buhrmester, C. C., Wenner, W. F. (1936) Arch. Otolaryng. **24,** 570.
Burch, G. E., DePasquale, N. P. (1962) Circ. Res. **10,** 105.
Burch, G. E., DePasquale, N. P. (1963) Amer. Heart J. **65,** 116.
Burn, J. H., Dale, H. H. (1926) J. Physiol. **61,** 185.
Burn, J. H., Rand, M. J. (1958) Brit. med. J. **1,** 903.
Burn, J. H., Leach, E. H., Rand, M. J., Thompson, J. W. (1959) J. Physiol. **148,** 332.
Burn, J. H., Rand, M. J. (1962) In: Advances in Pharmacology, Vol. 1. Ed. Garatini, S., Shore, P. A. (Academic Press, New York) p. 1.
Burnham, H. H. (1937) Ann. Otol. Rhinol. Laryng. **46,** 69.
Burnstein, M. Parrot, J. C. (1949) Soc. Biol., Paris **143,** 251.
Bussy, I. J. Le C. de, Lochem, J. J. van, Visser, A. K. (1942) Ned. T. Geneesk. **86,** 1273.
Carmichael, E. A., Fraser, F. R. (1933) Heart **16,** 263.
Carr, E .A. (1954) Pharmacol. Rev. **6,** 365.
Carrier, E. B. (1922) Amer. J. Physiol. **61,** 528.
Cate, H. J. ten (1954) Onderzoek bij Asthma-Patienten naar Overgevoeligheid voor Verstoven Allergeenextracten. (Thesis, Groningen).
Chambers, R., Zweifach, B. W. (1947) Physiol. Rev. **27,** 436.
Charms, B. L., Givertz, B., Toshihiko, I. (1962) Circulation **25,** 814.
Chobot, R., Dundy, H., Schaffer, N. (1940) J. Allergy **12,** 46.
Chorobski, J., Penfield, W. (1932) Arch. Neurol. Psychiat. **28,** 1257.
Christensen, K. (1934) Ann. Otol. Rhinol. Laryng. **43,** 1066.
Christensson, B., Ekwall, B. (1960) Acta allerg. **15,** 425.
Christopherson, J. B. (1933) Lancet **1,** 1.
Citron, K. M., Frankland, A. W., Sinclair, J. D. (1958) Thorax **13,** 229.
Clark, A. J. (1937) Handb. exp. Pharmacol. Suppl. **4,** 1.
Clark, E. R., Clark, E. L. (1935) Amer. J. Anat. **57,** 385.
Clark, E. R., Clark, E. L. (1936) Amer. J. Anat. **59,** 123.
Clarke, J. A., Rogers, H. L. (1937) Arch. Otolaryng. **25,** 124.
Clerici, E., Teatini, G. P.(1961) In: Allergy. Advances in Oto-Rhino-Laryngology. VII International Congress. Ed. Dishoeck, H. A. E. van. (Karger, Basel) p. 30.
Coakley, C. G. (1930) Manual of Diseases of the Nose and Throat (Churchill, London).
Coates, G. M., Ersner, M. S. (1930) Arch. Otolaryng. **11,** 158.
Coates, E. O., Bower, G. C., Reinstein, N. (1965) J. amer. med. Ass. **191,** 161.
Cobbold, A. F., Ginsburg, J., Paton, A. (1960) J. Physiol. **151,** 539.
Coca, A. F., Walzer, M., Thommen, A. A. (1931) Asthma and Hay Fever (Bailliére, Tindall & Cox, London).
Coca, A. F. (1931) J. Allergy **1,** 74.
Coca, A. F. (1934) J. Allergy **5,** 345.
Code, C. F., Macdonald, A. D. (1937) Lancet **2,** 730.
Code, C. F. (1937) J. Physiol. **89,** 257.
Code, C. F. (1939) Amer. J. Physiol. **127,** 78.

Code, C. F. (1952) Physiol. Rev. **32,** 47.
Code, C. F. (1952) Physiol. Rev. **37,** 42.
Code, C. F., Mitchell, R. G. (1957) J. Physiol. **136,** 449.
Code, C. F., Hurn, M. M., Mitchell, R. G. (1964) Mayo Clin. Proc. **39,** 715.
Cohen, M. B., Ecker, E. E., Breithart, J. R., Rudolph, J. A. (1930) J. Immunol. **18,** 419.
Cohen, M. B. (1939) J. Allergy **10,** 561.
Cohen, S. G., Kantor, M., Gatto, L. (1961) J. Allergy **32,** 214.
Cohen, S. G., Sapp, T. M. (1966) J. Allergy **36,** 415.
Coke, F. (1933) Colds and Hay Fever (Bailliére, Tindall & Cox, London).
Cole, P. (1954) J. Laryng. **68,** 295.
Colldahl, H., Lundin, G. (1952) Acta allerg. **5,** 37.
Colldahl, H. (1952) Acta allerg. **5,** 133.
Colldahl, H. (1952) Acta allerg. **5,** 143.
Colldahl, H. (1952) Acta allerg. **5,** 154.
Colldahl, H. (1959) Acta allerg. **14,** 42.
Colldahl, H. (1965) In: Abstracts of Symposia. The Sixth European Congress of Allergology, Stockholm. p. 5.
Collier, H. O. J., Holgate, J. A., Schachter, M., Shorley, P. G. (1959) J. Physiol. **149,** 54P.
Collier, H. O. J. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 5.
Collier, H. O. J., Holgate, J. A., Schachter, M., Shorley, P. G. (1960) Brit. J. Pharmacol. **15,** 290.
Collier, H. O .J., Shorley, P. G. (1960) Brit. J. Pharmacol. **15,** 601.
Collier, H. O. J. (1963) Ann. N. Y. Acad. Sci. **104,** 290.
Colmes, A., Rackemann, F. M. (1933) J. Allergy **4,** 473.
Comiskey, A. J. (1954) Ann. Allergy **12,** 637.
Comroe, J. H. (1939) Amer. J. Physiol. **127,** 116.
Comroe, J. H. (1952) Amer. J. Physiol. **171,** 715.
Comroe, J. H., Lingen, B. van, Straud, R. C., Roncoroni, A. (1953) Amer. J. Physiol. **173,** 379.
Comroe, J. H., Forster, R. E., Du Bois, A. B., Briscoe, W. A., Carlsen, E. (1962) The Lung. Clinical Physiology and Pulmonary Function Tests (2nd. ed.) (Year Book Publ., Chicago).
Comroe, J. H. (1965) Physiology of Respiration. (Year Book Medical Publ., Chicago).
Connell, J. T. (1966) J. Allergy, **37,** 127.
Connor, P. K., Kinard, S. A., Ford, R., McConn, R. G., Mogar, J., (1957) Geriatrics **12,** 185.
Cooke, R. A., Veer, A. van der (1916) J. Immunol. **1,** 201.
Cooke, R. A. (1918) N. Y. St. J. Med. **107,** 577.
Cooke, R. A. (1922) J. Immunol. 7, 119.
Cooke, R. A. (1922) J. Immunol. **7,** 147.
Cooke, R. A. (1930) Laryngoscope **40,** 210.
Cooke, R. A. (1932) Amer. J. med. Sci. **183,** 309.
Cookson, D. U., Reed, C. E. (1963) Amer. Rev. resp. Dis. **88,** 636.
Copley, A. L., Tulusca, V. (1963) In: Mode of Action of Drugs. First International Pharmacological Meeting, 1961, Vol. 9, Part 1. Ed. Uvnäs, B. (Pergamon Press, Oxford) p. 67.
Cottle, M. H. (1960) Corrective Surgery Nasal Septum and External Pyramid. (American Rhinologic Society, Chicago).
Cottle, M. H. (1958) J. int. Coll. Surg. **29,** 472.
Cottle, M. H. (1960) Arch. Otolaryng. **72,** 11.
Cottle, M. H., Loring, R. M., Gaynon, I. E. (1963) Int. Rhinol. **1,** 23.
Courtade, A. (1902) La respiration est-elle exclusivement nasale ou buccale ou peut-elle être mixte. Dans Soc. de Médicine et de Chir. pratique. Paris, 17 janv. (Quoted by Itié 1909).

Cramer, I. I. (1957) Ann. Otol. Rhinol. Laryng. **66,** 327.
Crawford, L. V. (1960) Ann. Allergy **18,** 59.
Curry, J. J. (1946) J. clin. Invest. **25,** 785.
Curry, J. J. (1946) J. clin. Invest. **25,** 792.
Curry, J. J. (1947) J. clin. Invest. **26,** 430.
Curtis, D. R., Ryall, R. W., Watkins, J. C. (1965) In: Pharmacology and Cholinergic and Adrenergic Transmission. Second International Pharmacological Meeting, Vol. 3. Ed. Koelle, G. B., Douglas, W. W., Carlsson, A., Trcka, V. (Pergamon Press, Oxford) p. 137.
Dale, H. H., Laidlaw, P. P. (1910) J. Physiol. **41,** 318.
Dale, H. H., Laidlaw, P. P. (1911) J. Physiol. **43,** 182.
Dale, H. H. (1913) J. Pharmacol. exp. Ther. **4,** 167.
Dale, H. H. (1914) J. Pharmacol. exp. Ther. **6,** 147.
Dale, H. H., Laidlaw, P. P. (1918) J. Physiol. **52,** 355.
Dale, H. H., Richards, A. N. (1918) J. Physiol. **52,** 110.
Dale, H. H. (1929) Lancet **1,** 1285.
Dale, H. H. (1948) Brit. med. J. **II,** 281.
Dalhamn, T., Rhodin, J. (1956) Brit. J. industr. Med. **13,** 110.
Dalton, D. J. (1949) Lancet **2,** 607.
Daly de Burgh, I., Foggie, P., Ludány, G. von (1937) Quart. J. exp. Physiol. 26, 235.
Daly de Burgh, I. (1958) Quart. J. exp. Physiol. **43,** 2.
Daly de Burgh, M. (1954) Pharmacol. Rev. **6,** 79.
Daly de Burgh, M. (1957) Brit. J. Pharmacol. **12,** 504.
Danfelt, C. J. (1948) Acta med. scand. **239,** 320.
Danforth, H. B. (1964) Laryngoscope **74,** 115.
Davis, D. L., Hertzman, A. B. (1957) Ann. Otol. Rhinol. Laryng. **66,** 622.
Dawes, J. D. K., Prichard, M. M. L. (1953) J. Anat. **87,** 311.
Dawson, W. T., Newman, S. P. (1931) J. amer. med. Ass. **97,** 930.
Dean, L. W., Linton, L. D., Linton C. S. (1935) Ann. Otol. Rhinol. Laryng. **44,** 317.
Dean, L. W., (1942) J. amer. med. Ass. **99,** 542.
Dekker, E., Groen, J. (1957) Lancet **1,** 1064.
Dekker, E., DeFares, J. G., Heemstra, H. (1958) J. appl. Physiol. **13,** 35.
Demling, L., Gromotka, R. Bünte, H. (1959) Z. Kreisl.Forschr. **48,** 225.
Dews, P. B., Wnuck, A. L., Fanelli, R. V., Light, A. E., Tallaben, J. A., Norton, S., Ellis, L. H., Beer, E. J. de (1953) J. Pharmacol. exp. Ther. **107,** 1.
Dintenfass, A. (1956) Arch. Otolaryng. **64,** 171.
Dishoeck, H. A. E. van (1936) Acta oto-laryng., Stockh. **24,** 494.
Dishoeck, H. A. E. van (1940) Arch. Otolaryng. **4,** 588.
Dishoeck, H. A. E. van (1941) Ned. T. Geneesk. **85,** 2564.
Dishoeck, H. A. E. van, Klein, S. P. (1942) Ned. T. Geneesk. **86,** 2666.
Dishoeck, H. A. E. van (1942) Acta oto-laryng., Stockh. **30,** 431.
Dishoeck, H. A. E. van, Klein, S. P. (1943) Ned. T. Geneesk. **87,** 1040.
Dishoeck, H. A. E. van (1950) Ned. T. Geneesk. **94,** 224.
Dishoeck, H. A. E. van (1950) Ned. T. Geneesk. **94,** 2288.
Dishoeck, H. A. E. van (1952) Ned. T. Geneesk. **96,** 1630.
Dishoeck, H. A. E. van (1954) Ned. T. Geneesk. **98,** 2894.
Dishoeck, H. A. E. van (1954) In: Leerboek der Ziekten van Oren, Neus, Mond, Keel, Slokdarm en Lagere Luchtwegen. Ed. Gerlings, P. G. (F. Bohn N.V., Haarlem) p. 336.
Dishoeck, H. A. E. van (1961) In: Allergy. Advances in Oto-Rhino-Laryngology. VII International Congress. Ed. Dishoeck, H. A. E. van. (Karger, Basel) p. 1.
Dishoeck, H. A .E. van, Lier, L. A. J. van (1963) Int. Rhinol. **1,** 52.
Dishoeck, H. A. E. van (1965) Int. Rhinol. **3,** 19.
Dixon, W. E., Brodie, T. G. (1903) J. Physiol. **29,** 97.
Dixon, W. E., Ranson, F. (1912) J. Physiol. **45,** 413.

Dixon, W. E., Hoyle, J. C. (1930) J. Physiol. **70,** 1.
Doeleman, F., Koekenheim, L. (1957) Sociaal-Geneeskundige Studies over Asthma Bronchiale. (Van Gorcum, Assen).
Donleben, P. G. (1959) Studies on Lungmechanics. (Thesis, Groningen).
Douglas, W. W., Toh, C. C. (1953) J. Physiol. **120,** 311.
Dragstedt, C. A., Gebauer-Fuelnegg, E. (1932) Amer. J .Physiol. **102,** 512.
Dragstedt, C. A., Ramirez de Arellano, M., Lawton, A. H. (1940) Science **91,** 617.
Dragstedt, C. A. (1941) Physiol. Rev. **21,** 563.
Dragstedt, C. A. (1947) J. amer. med. Ass. **135,** 133.
Dragstedt, C. A. (1950) Ann. N.Y. Acad. Sci. **50,** 1039.
Drettner, B. (1961) Acta oto-laryng., Stockh. Suppl. 166.
Duff, F., Greenfield, A. D. M., Shepherd, J. T., Thompson, I. D. (1953) J. Physiol. **120,** 160.
Dufton, A. F., Bedford, T. (1933) J. Hyg. **33,** 476.
Duchaine, J., Spapen, R. (1959) In: Inhalatietests en Inhalatie Therapie. Verslag van een Vergadering van de Belgische en Nederlandse Verenigingen voor Allergie. (H. E. Stenfert Kroese, Leiden) p. 85.
Duke, W. W. (1924) J. amer. med. Ass. **83,** 3.
Duke, W. W. (1925) Allergy, Asthma, Hay Fever, Urticaria and Allied Manifestations of Reaction. (Mosby, St. Louis).
Duke, H. N., Lee, G. de J. (1963) Brit. med. Bull. **19,** 71.
Dunbar, W. P. (1903) Münch. med. Wschr. **50,** 924.
Dunér, H. B., Pernow, B. (1958) Acta med. scand. **161,** 361.
Dunér, H. B., Pernow, B. (1960) Acta med. scand. **168,** 307.
Dunér, H., Pernow, B., Sterky, G. (1960) Acta allerg. **15,** 417.
Durham, O. C. (1928) J. Lab. clin. Med. **13,** 967.
Durham, O. C. (1930) J. Allergy **1,** 12.
Durham, O. C. (1931) J. Allergy, **2,** 258.
Durham, O. C. (1933) J. Allergy, **4,** 104.
Durham, O. C. (1933) J. amer. med. Ass. **100,** 1846.
Durham, O. C. (1935) J. Allergy, **6,** 128.
Durward, A., Lord, O. C., Polson, C. J. (1945) J. Laryng. **60,** 461.
Dutta, N. K., Narayanan, K. G. A. (1952) Nature **169,** 1064.
Dzury, D. S., Cohen, S. G. (1958) J. Allergy **29,** 340.
Edery, H. (1965) Brit. J. Pharmacol. **24,** 485.
Editorial (1952) Ann. Allergy **10,** 343.
Editorial (1952) Ann. Allergy **10,** 775.
Edwards, H. E. (1952) Int. Corr. Soc. Allergy **15,** 21.
Efron, B. G., Penfound, W. T. (1930) J. Allergy **2,** 43.
Eggston, A. A., Wolff, D. (1947) Histopathology of the Ear, Nose and Throat. (Williams & Wilkins, Baltimore).
Egmond, A. A. J. van (1955) Ned. T. Geneesk. **99,** 3330.
Ehrlich, P. (1879) Arch. Anat. Physiol. p. 571.
Eichler, O., Barfuss, F. (1940) Arch. exp. Path. Pharmakol. **195,** 245.
Eidinger, D., Raff, M., Rose, B. (1962) Nature **196,** 683.
Elliotson, J. (1830) Lancet **2,** 370.
Elliotson, J. (1831) London med. Gaz. **8,** 411.
Elliott, D. F., Horton, E. W., Lewis, G. P. (1960) J. Physiol. **153,** 473.
Elliott, D. F., Horton, E. W., Lewis, G. P. (1960) J. Physiol. **150,** 6P.
Elliott, D. F. (1963) In: Mode of Action of Drugs. First International Pharmacological Meeting, 1961, Vol. 9, Part 1. Ed. Uvnäs, B. (Pergamon Press, Oxford) p. 25.
Ellis, L. B., Weiss, S. (1932) J. Pharmacol. **44,** 235.
El Mehairy, M. M., El Tarabishy, N., Nabih, A. M., Nassar, I. (1965) Ann. Allergy **23,** 93.
Erspamer, V. (1952) Nature **169,** 800.
Erspamer, V. (1952) Nature **170,** 282.

Erspamer, V. (1954) Pharmacol. Rev. **6,** 425.
Erspamer, V. (1966) In: Handbook of Experimental Pharmacology, Vol. 19: 5-Hydroxytryptamine and Related Indolealkylamines. Ed. Eichler, O., Farah, A., Erspamer, V. (Springer-Verlag, Berlin) p. 360.
Escat, E. (1908) Ann. Mal. Or. **34,** 2, 207.
Euler, U. S. von (1932) J. Physiol. **74,** 271.
Euler, U. S. von (1949) Acta physiol. scand. **19,** 85.
Evans, C. L. (1956) Principles of Human Physiology. (Churchill, London).
Evans, W. H. (1955) Ann. Allergy **13,** 99.
Everett, H. C. (1964) Neurology **14,** 483.
Ewins, A. J. V. (1914) Biochem. J. **8,** 44.
Eyermann, C. H. (1927) Ann. Otol. Rhinol. Laryng. **36,** 808.
Eyermann, C. H. (1930) J. Allergy **1,** 350.
Fabricant, N. D. (1942) Nasal Medication. (Williams & Wilkins, Baltimore).
Fabricant, N. D., Neffson, A. H. (1960) Amer. J. med. Sci. **239,** 498.
Faille, R. L. Baart de la (1951) Ned. T. Geneesk. **95,** 3066.
Fastman, N. S., Glazer, J. (1963) Ann. Allergy **21,** 1.
Feinberg, S. M., Steinberg, M. J. (1933) J. Allergy **5,** 19.
Feinberg, S. M. (1934) J. amer. med. Ass. **102,** 1557.
Feinberg, S. M. (1939) Arch. Derm. Syph. **40,** 200.
Feinberg, S. M. (1946) Allergy in Practice. (Year Book Publ., Chicago).
Feinberg, S. M., Stier, R. A., Grater, W. C. (1952) J. Allergy **23,** 387.
Feinberg, S. M., Sternberger, L. A. (1955) J. Allergy **26,** 170.
Feldberg, W., Vartiainen, A. (1935) J. Physiol. **83,** 103.
Feldberg, W., Kellaway, C. H. (1937) J. Physiol. **90,** 257.
Feldberg, W., Keogh, E. V. (1937) J. Physiol. **90,** 280.
Feldberg, W., O'Connar, W. J. (1937) J. Physiol. **90,** 288 .
Feldberg, W., Miles, A. A. (1951) J. Physiol. **114,** 34P.
Feldberg, W., Paton, W. D. M. (1951) J. Physiol. **114,** 490.
Feldberg, W., Schachter, M. (1952) J. Physiol. **118,** 124.
Feldberg, W., Miles, A. A. (1953) J. Physiol. **120,** 205.
Feldberg, W., Talesnik, J. (1953) J. Physiol. **120,** 550.
Feldberg, W., Smith, A. N. (1953) J. Physiol. **122,** 62P.
Feldberg, W. (1953) Proc. roy. Soc. Med. **46,** 256.
Feldberg, W. (1954) Pharmacol. Rev. **6,** 85.
Feldberg, W., Mongar, J. L. (1954) Brit. J. Pharmacol. **9,** 197.
Feldberg, W., Lecomte, J. (1955) Brit. J. Pharmacol. **10,** 254.
Feldberg, W. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 4.
Fenton, R. A., Larsell, O. (1934) Arch. Otolaryng. **19,** 183.
Fernex, M., Fernex, S. (1966) Schweiz. med. Wschr. **96,** 46.
Ferris, B. G., Anderson, D. O. (1962) Amer. Rev. resp. Dis. **85,** 165.
Field, M. E., Drinker, C. K. (1930) Amer. J. Physiol. **93,** 138.
Finck, H. P. (1927) Laryngoscope **37,** 783.
Fisher, A. M., Christie, H. (1962) Brit. J. industr. Med. **19,** 236.
Fishman, A. P. (1963) In: Handbook of Physiology Section 2: Circulation II. Ed. Hamilton, W. F., Dow, P. (Williams & Wilkins, Baltimore) p. 1667.
Fleisch, A. (1930) Pflügers Arch. ges. Physiol. **228,** 351.
Fleming, A. (1928) J. Laryng. **43,** 385.
Fletcher, C. M. (1959) Amer. Rev. resp. Dis. **80,** 483.
Fletcher, C. M. (1959) Brit. med. J. **II,** 257.
Florey, H., Carleton, H. M., Wells, A. Q. (1932) Brit. J. exp. Path. **13,** 269.
Florey, H. (1962) General Pathology (Lloyd-Luke, London) p. 21.
Foggie, P. (1937) Quart. J. exp. Physiol. **26,** 225.
Forman, J. (1934) Arch. Otolaryng. **19,** 367.
Forman, J. (1934) Ann. Otol. Rhinol. Laryng. **43,** 279.
Forster, R. E. (1957) Physiol. Rev. **37,** 391.
Fowler, E. P. (1943) Arch. Otolaryng. **37,** 710.

Fowler, N. O. (1960) Amer. J. Med. **28,** 927.
Fowler, J. W., Lowell, F. C. (1966) J. Allergy **37,** 19.
Fox, L. A. (1959) Ann. Allergy **17,** 768.
Fox, N., Fabricant, N. (1933) Arch. Otolaryng. **18,** 181.
Fox, R. H., Hilton, S. M. (1958) J. Physiol. **142,** 219.
Fox, R. H., Goldsmith, R., Kidd, D. J., Lewis, G. P. (1960) J. Physiol. **154,** 16P.
Fox, R. H., Goldsmith, R., Kidd, D. J., Lewis, G. P. (1961) J. Physiol. **157,** 589.
Foy, R. (1910) Ann. Mal. Or. **36,** 1, 130.
Frank, D. E., MacLaren, W. R. (1954) Ann. Allergy **12,** 289.
Frank, D. E., MacLaren, W. R. (1957) Ann. Allergy **15,** 261.
Frank, D. E. (1959) Ann. Allergy **17,** 200.
Franklin, K. J. (1932) J. Physiol. **75,** 471.
Franssen, M. G. C. (1957) Ned. T. Geneesk. **101,** 847.
Fraser, P. J. (1957) Brit. J. Pharmacol. **12,** 47.
Freitas, F. M. de, Faraco, E. Z., Azevedo, D. F. de (1964) Circulation **29,** 66.
Frey, E. K., Kraut, H. (1928) Arch. exp. Path. Pharmakol. **133,** 1.
Frey, E. K. (1963) Ann. N.Y. Acad. Sci. **104,** 90.
Freyburger, W. A., Graham, B. E., Rapport, M. M., Seay, P. H. Govier, W. M., Swoap, O. F., Brook, M. J. van der (1952) J. Pharmacol. exp. Ther. **105,** 80.
Frick, O. L. (1966) Ann. Allergy **24,** 95.
Fries, J. H. (1959) Ann. Allergy **17,** 1.
Fritts, H. W., Harris, P., Clauss, R. H., Odell, J. E., Cournand, A. (1958) J. clin. Invest. **37,** 99.
Furstenberg, A. C. (1963) Ann. Otol. Rhinol. Laryng. **72,** 1076.
Fürth, O. von (1900) Z. phys. Chem. **29,** 105.
Gaddum, J. H., Holtz, P. (1933) J. Physiol. **77,** 139.
Gaddum, J. H. (1953) Pharmacol. Rev. **5,** 87.
Gaddum, J. H. (1953) J. Physiol. **119,** 363.
Gaddum, J. H., Hebb, C. O., Silver, A., Swan, A. A. B. (1953) Quart. J. exp. Physiol. **38,** 255.
Gaddum, J. H. (1953) Pharmacology (Oxford University Press, London).
Gaddum, J. H. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 36 & 285.
Gaddum, J. H. (1957) Pharmacol. Rev. **9,** 211.
Gaddum, J. H. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 195.
Gaillard, G. E., Schellin, R., Mayers, R. A. (1962) Ann. Allergy **20,** 81.
Gaillard, G. E., Schellin, R., Mayers, R. A. (1963) Ann. Allergy **21,** 145.
Gaillard, G. E., Mayers, R. A., Schellin, R. (1963) Ann. Allergy **21,** 210.
Galen, C. (1964) Corpus Medicorum Graecorum, Supplementum V. Ed. Kollesch, J. (Academie-Verlag, Berlin).
Galen, C. Works of Kühn's edition (1893), Teubner, Leipzig.
Garattini, S., Valzelli, L. (1965) Serotonin. (Elsevier, Amsterdam).
Gardner, W. J., Stowell, A., Dutlinger, R. (1947) J. Neurosurg. **4,** 105.
Garel, D. (1906) Das Heufieber. (München).
Gebauer-Fuelnegg, E., Dragstedt, C. A. (1932) Amer. J. Physiol. **102,** 520.
Gelfand, H. H. (1930) J. Allergy **1,** 223.
Gelfand, H. H. (1932) Arch. Otolaryng. **16,** 550.
Gelfand, H. H. (1963) Ann. Allergy **21,** 150.
Gell, P. G. H. (1961) Int. Arch. Allergy **18,** 39.
Geubelle, F., Monard, Y., Abrassart, C. (1965) In: Abstracts. Sixth European Congress of Allergology, Stockholm (Ohlssons Boktryckeri, Lund) p. 37.
Geus, J. P., Heuting, J. E. de, Visser, P. (1956) Acta physiol. pharmacol. neerl. **5,** 216.
Gilbert, R. P., Kuida, H., Hinslaw, L. B., Visscher, M. (1957) Clin. res. Proc. **5,** 225.
Gilbert, R. P., Hinslaw, L. B., Kuida, H., Visscher, M., (1958) Amer. J. Physiol. **194,** 165.

Giles, A. (1961) Ann. Allergy **19,** 511.
Gill, J. R., Melmon, K. L., Gillespie, L., Bartter, C. (1965) Amer. J. Physiol. **209,** 844.
Gillespie, M. (1930) Brit. med. J. **I,** 1139.
Ginsburg, J., Cobbold, A. F. (1960) In: Adrenergic Mechanisms. Ciba Foundation Symposium. Ed. Vane, J. R., Wolstenholme, G. E. W., O'Connor, M. (Churchill, London) p. 173.
Ginzel, K. H., Kottegoda, S. R. (1953) Quart. J. exp. Physiol. **38,** 225.
Ginzel, K. H., Kottegoda, S. R. (1954) J. Physiol. **123,** 277.
Ginzel, K. H. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 131.
Glaser, G., Merritt, H. H. (1952) J. amer. med. Ass. **148,** 898.
Glatzel, (1901) Ther. Gegenw. **42,** 348 & 501.
Glover, W. E., Greenfield, A. D. M., Kidd, B. S. L. Whelan, R. F. (1958) J. Physiol. **140,** 113.
Gocke, T. M., Duffy, B. J. (1962) Arch. intern. Med. **110,** 605.
Godlowski, Z. Z. (1948) Brit. J. exp. Path. **29,** 511.
Godlowski, Z. Z. (1948) Brit. med. J. **I,** 46.
Goldberg, L. I. (1959) Ann. N.Y. Acad. Sci. **80,** 639.
Goldfarb, A. A., Venutolo, F. (1963) Ann. Allergy **21,** 667.
Golding-Wood, P. H. (1961) J. Laryng. **75,** 232.
Golding-Wood, P. H. (1962) J. Laryng. **76,** 969.
Golding-Wood, P. H. (1963) Int. Rhinol. **1,** 188.
Goodale, J. L. (1916) Boston med. sci. J. **175,** 181.
Goodale, J. L. (1922) Ann. Otol. Rhinol. Laryng. **31,** 882.
Goodman, L. S., Gilman, A. (1956) The Pharmacological Basis of Therapeutics (MacMillan, New York).
Gordon, B. L. (1949) Medicine Throughout Antiquity (Davis, Philadelphia).
Gordon, W. (1829) Med. Gaz. **4,** 266.
Goslings, W. R. O. (1959) Ned. T. Geneesk. **103,** 1683.
Goth, A. (1961) Medical Pharmacology (Mosby, St. Louis).
Gottlieb, M. J. (1927) Laryngoscope **37,** 719.
Gottlieb, P. M. (1954) Ann. Allergy **12,** 456.
Gottlieb, P. M. (1955) Ann. Allergy **13,** 423.
Grab, D., Winkenwerder, W. L., Schoensich, E. H. (1952) Bull. Johns Hopk. Hosp. **90,** 301.
Graham, H. T., Lowry, O. H., Wheelwright, F. L. M. A., Parish, H. H. (1955) Blood **10,** 467.
Grant, R. T. (1930) Heart **15,** 281.
Grant, R. T., Bland, E. F. (1931) Heart **15,** 385.
Grant, W. M. (1950) Arch. Ophthal. **44,** 362.
Green, H. D. (1954) In: Pharmacology in Medicine. Ed. Drill, V. A. (McGraw-Hill Book Co., New York).
Green, M. A. (1962) Ann. Allergy **20,** 193.
Green, M., Solomon, D. (1962) Ann. Allergy **20,** 373.
Greenstein, N. M. (1955) J. amer. med. Ass. **157,** 1153.
Greenwald, I. (1954) Arch. industr. Med. **10,** 455.
Griebel, C. R. (1938) Klin. Wschr. **17,** 164.
Grollman, A. (1960) Pharmacology and Therapeutics (Lea & Febiger, Philadelphia).
Gross, P. (1963) Arch. environm. Hlth. **6,** 42.
Grove, R. C. (1951) J. Allergy **22,** 550.
Grove, W. E. (1927) Arch. Otolaryng. **6,** 237.
Guerrant, J. L., Pearsal, H. R. (1960) Ann. Allergy **18,** 1226.
Guggenheim, M., Löffler, W. (1916) Biochem. Z. **72,** 303.
Gurney, C. W., Cryst, S. (1957) Ann. Allergy **15,** 367.
Haddy, F. J., Fleishmann, M., Emanuel, D. A. (1957) Circ. Res. **5,** 247.

Haddy, F. J., Gordon, P., Emanuel, D. A. (1959) Circ. Res. **7**, 123.
Haddy, F. J. (1960) Fed. Proc. **19**, 56.
Haddy, F. J., Molnar, J. I., Borden, C. W., Texter, E. C. (1962) Circulation **25**, 239.
Hagen, P., Barrnett, R. J., Lee, F. L. (1959) J. Pharmacol. exp. Ther. **126**, 91.
Hager, A. (1952) Z. Laryng. Rhinol. **31**, 195.
Hahn, N. (1962) Arzneimittelforsch. **12**, 975.
Hajós, M. K. (1962) Acta allerg. **17**, 358.
Hal, I. v. d. (1947) Ned. T. Geneesk. **91**, 2746.
Hald, E. (1943) Acta oto-laryng., Stockh. **31**, 23.
Hallo, H. C. (1954) Ned. T. Geneesk **98**, 2642.
Hallo, H. C. (1955) Ned. T. Geneesk. **99**, 3097.
Halmágyi, D. F. J., Colebatch, H. J. H. (1963) J. clin. Invest. **40**, 1785.
Halmagyi, D. F. J., Colebatch, H. J. H., Starzecki, B., McRae, J. (1963) Amer. Heart J. **65**, 208.
Halpern, B. N. (1953) In: The Mechanism of Inflammation (Acta Inc., Montreal) p. 228.
Halpern, B. N. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 92.
Halpern, B. N. (1960) Histamine et Allergie. In: Acquisitions Médicales Recentes (Flammarion, Paris).
Halpern, B. N. (1960) In: Fundamentals of Modern Allergy. Ed. Prigal, S. J. (McGraw-Hill Book Co., New York) p. 27.
Halpern, S. R., Holman, J., Whittaker, C. (1961) Ann. Allergy **19**, 1407.
Halpin, L. J. (1957) Ann. Allergy **15**, 315.
Ham, A. W. (1965) Histology (Pitman, London).
Hansel, F. K. (1924) J. amer. med. Ass. **82**, 15.
Hansel, F. K. (1929) J. Allergy **1**, 43.
Hansel, F. K. (1934) J. Allergy **5**, 357.
Hansel, F. K. (1936) Allergy of the Nose and Paranasal Sinuses (Kimpton, London).
Hansel, F. K. (1953) Clinical Allergy. (Mosby, St. Louis).
Hansen, K. (1954) acta allerg. **7**, 163.
Hanzlik, P. J. (1925) Amer. J. Physiol. **71**, 558.
Harkins, H. P. (1954) Arch. Otolaryng. **59**, 531.
Harkness, G. F. (1934) Ann. Otol. Rhinol. Laryng. **43**, 146.
Harper, A. R. (1959) J. Laryng. **73**, 21.
Harris, A. S. (1939) Ann. Otol. Rhinol. Laryng. **48**, 311.
Harris, L. H. (1941) J. Allergy **12**, 279.
Harris, M. C., Shure, N. (1957) Practical Allergy (Butterworths, London).
Harris, P. (1957) Brit. Heart J. **19**, 272.
Harris, P., Fritts, H. W., Cournand, A. (1960) Circulation **21**, 1134.
Hartman, M. M. (1947) Calif. Med. **66**, 242.
Hartman, M. M. (1956) Amer. J. Med. **21**, 85.
Hartman, M. M. (1957) Ann. Allergy **15**, 391.
Hartz, H. J. (1909) Ann. Otol. Rhinol. Laryng. **18**, 709.
Hashimoto, K., Shigei, T., Imai, S., Saito, Y., Yago, N., Uei, I., Clark, R. E. (1960) Amer. J. Physiol. **198**, 965.
Hawkins, D. F., Schild, H. O. (1951) Brit. J. Pharmacol. **6**, 682.
Haworth, E., McDonald, A. D. (1937) J. Hyg. **37**, 234.
Hayek, H. (1962) In: Pulmonary Structure and Function. Ciba Foundation Symposium. Ed. Reuck, A. V. S. de, O'Connor, M. (Churchill, London) p. 99.
Hebb, C. O. (1954) Pharmacol. Rev. **6**, 39.
Hebb, C. O. (1957) Physiol. Rev. **37**, 196.
Heetderks, D. R. (1927) Amer. J. med. Sci. **174**, 231.
Heimann, H. (1963) Ann. Allergy **21**, 396.

Hellman, E. (1952) J. amer. med. Ass. **149,** 1393.
Hellman, K. (1926) Z. Laryng. Rhinol. **15,** 1.
Henderson, L. L., Code, C. F., Roth, G. M. (1958) J. Allergy **29,** 122.
Herck. (1844) (Quoted by Bray, 1931).
Herschfus, J. A., Bresnick, E., Segal, M. S. (1953) Amer. J. Med. **14,** 34.
Herxheimer, H. (1949) J. Physiol. **109,** 4P.
Herxheimer, H., Short, R. H. D. (1949) Brit. J. Pharmacol. **4,** 311.
Herxheimer, H. (1951) Dtsch. med. Wschr. **76,** 1171.
Herxheimer, H. (1952) J. Allergy Abstracts **23,** 33.
Herxheimer, H. (1952) Int. Arch. Allergy **3,** 323.
Herxheimer, H., Prior, F. N. (1952) Int. Arch. Allergy **3,** 189.
Herxheimer, H. (1953) J. Physiol. **120,** 65P.
Herxheimer, H. (1953) J. Physiol. **122,** 49P.
Herxheimer, H., McInroy, P., Sutton, F. N., Utidjian, H. L., Utidjian, H. M. D. (1954) Acta allerg. **7,** 380.
Herxheimer, H. (1955) J. Physiol. **128,** 435.
Herxheimer, H. (1955) Brit. J. Pharmacol. **10,** 160.
Herxheimer, H. (1956) Arch. int. Pharmacodyn. **106,** 371.
Herxheimer, H. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 163.
Herxheimer, H., Schachter, M. (1959) J. Physiol. **145,** 34P.
Herxheimer, H., Schachter, M. (1959) Nature **183,** 1510.
Herxheimer, H., Stresemann, E. (1961) J. Physiol. **158,** 38P.
Heymans, C., Heuvel-Heymans, G. van den (1953) Arch. int. Pharmacodyn. **93,** 95.
Higbee, D. R. (1936) Ann. Otol. Rhinol. Laryng. **45,** 385.
Higginbotham, R. D. (1966) In: Handbook of Experimental Pharmacology, Vol. 18. Histamine and Anti-Histaminics. Part I: Histamine, Its Chemistry, Metabolism and Physiological and Pharmacological Actions. Ed. Eichler, O., Farah, A., Rocha e Silva, M. (Springer-Verlag, Berlin) p. 638.
Higgins, I. T. T. (1957) Brit. med. J. **II,** 1198.
Higgins, I. T. T., Cochran, J. B. (1958) Tubercle **39,** 296.
Hill, L. (1932) J. Physiol. **74,** 1.
Hill, L. (1933) Brit. med. J. **i,** 1096.
Hilding, A. C. (1957) Amer. J. Physiol. **191,** 404.
Hilding, A. C. (1964) Ann. Otol. Rhinol. Laryng. **73,** 358.
Hillis, B. R. (1952) Lancet **1,** 1230.
Hilton, S. M., Lewis, G. P. (1956) J. Physiol. **134,** 471.
Hilton, S. M. (1963) Ann. N.Y. Acad. Sci. **104,** 275.
Hinderer, K. H. (1961) Ann. Allergy **19,** 147.
Hinderer, K. H. (1964) Int. Rhinol. **2,** 53.
Hirsch, J. G. (1965) In: The Inflammatory Process. Ed. Zweifach, B. W., Grant, L. McCluskey, R. T. (Academic Press, New York) p. 245.
Hlavácek, V. (1961) In: Allergy. Advances in Oto-Rhino-Laryngology. VII International Congress. Ed. Dishoeck, H. A. E. van, (Karger, Basel) p. 65.
Hofer, G. (1963) Acta oto-laryng., Stockh. Suppl. 183.
Högberg, B., Uvnäs, B. (1957) Acta chem. scand. **11,** 1092.
Holdstock, D. J., Mathias, A. P., Schachter, M. (1957) Brit. J. Pharmacol. **12,** 149.
Holland, W. W., Spicer, C. C., Wilson, J. M. G. (1961) Brit. med. J. **II,** 338.
Holland, W. W., Reid, D. D. (1965) Lancet **1,** 445.
Holmes, T. H., Goodell, H., Wolf, S., Wolff, H. G. (1950) The Nose, An Experimental Study of Reactions within the Nose in Human Subjects during Varying Life Experiences. (Thomas, Springfield).
Holton, P., Rand, M. J. (1962) Brit. J. Pharmacol. **19,** 513.
Hooker, D. R. (1920) Amer. J. Physiol. **54,** 30.
Hopkins, M. (1931) J. Allergy **2,** 145.
Hopp, E. S., Burn, H. F. (1958) Ann. Otol. Rhinol. Laryng. **67,** 480.

Horton, B. T., Brown, G. E., Roth, G. M. (1936) J. amer. med. Ass. **107,** 1263.
House, E. L., Pansky, B. (1960) A Functional Approach to Neuro-Anatomie (McGraw-Hill Book Co., New York).
Huber, H. L., Koessler, K. K. (1922) Arch. intern. Med. **30,** 689.
Huber, H. L. (1931) J. Allergy **2,** 48.
Huber, H. L., Harsch, G. F. (1934) J. Allergy **5,** 432.
Hubert, L. (1924) Ann. Otol. Rhinol. Laryng. **33,** 824.
Hubert, L. (1930) Arch. Otolaryng. **12,** 78.
Huhti, E. (1965) Acta tuberc. scand. Suppl. 61.
Humphrey, J. H., Jaques, R. (1953) J. Physiol. **119,** 43P.
Humphrey, J. H., Jaques, R. (1954) J. Physiol. **124,** 305.
Humphrey, J. H., Jaques, R. (1955) J. Physiol. **128,** 9.
Hunt, R. (1899—1900) Amer. J. Physiol. **18,** 19.
Hunt, R., Taveau, R. de M. (1906) Brit. med. J. **I,** 1788.
Hurtado, A., Kaltreider, N. L. (1934) J. clin. Invest. **13,** 1053.
Hurwitz, S. H. (1930) J. Allergy **1,** 245.
Ichizaki, T., Yokohari, R., Araki, H., Miyanto, T., Hirose, S., Kape, J., Otsuka, S., Makino, S. (1962) Jap. J. Allergy **11,** 336 [In: Abstracts of J. Allergy (1962) **33,** 35].
Inderbitzin, T. (1961) Int. Arch. Allergy **18,** 85.
Ingelstedt, S., Ivstam, B. (1949) Acta oto-laryng. **37,** 446 & 451.
Ingelstedt, S., Toremalm, N. G. (1960) Acta oto-laryng. Suppl. **158,** 81.
Ingersoll, J. M. (1922) Trans amer. otol. Soc. **28,** 162.
Ingersoll, J. M. (1908) Ann. Otol. Rhinol. Laryng. **17,** 901.
Innes, I. R. (1962) Brit. J. Pharmacol. **19,** 427.
Innes, I. R., Nickerson, M. (1965) In: The Pharmacological Basis of Therapeutics. Ed. Goodman, L. S., Gilman, A. (MacMillan, New York) p. 477.
Itié, R. (1909) Recherches de Rhinométrie Clinique. Contribution à l'étude de la Respiration Nasale. (Thesis, Toulouse) [Quoted by Uddströmer, 1940].
Itkin, I. H., Anand, S., Yau, M., Middlebrook, G. (1963) J. Allergy **34,** 97.
Jackson, C., Jackson, C. L. (1945) Diseases of the Nose, Throat and Ear, including Bronchoscopy and Esophagoscopy. (Saunders, Philadelphia).
Jackson, D. E. (1942) Ann. Otol. Rhinol. Laryng. **51,** 973.
Jakowska, S. (1963) Ann. N.Y. Acad. Sci. **106,** 157.
James, J. A. (1965) In: Diseases of the Ear, Nose and Throat, Vol. I. Ed. Scott-Brown, W. G., Ballantyne, J., Groves, J. (Butterworths, London) p. 353.
Jamieson, D. (1962) Brit. J. Pharmacol. **19,** 286.
Johnson, K. J. (1963) Ann. Allergy **21,** 650.
Johnson, J. J., Sheldon, J. M., Mathews, K. P., Lovell, R. G. (1952) J. Allergy **23,** 365.
Johnson, M. C., Goldstein, D. W. (1932) Arch. Otolaryng. **16,** 808.
Jones, J. V. (1952) Brit. J. Pharmacol. **7,** 450.
Jones, M. D. (1952) Arch. Otolaryng. **55,** 261.
Jongh, S. E. de (1959) Inleiding tot de Algemene Farmacologie (Noord-Hollandsche Uitgeversmaatschappij, Amsterdam).
Josey, A. L., Lawrence, J. S. (1932) Folia haemat. **48,** 313.
Josey, A. L., Lawrence, J. S. (1932) Folia haemat. **48,** 330.
Juhlin-Dannfelt, C. (1950) Acta med. scand. **138,** Suppl. 239, 320.
Kabins, S. A., Molina, C., Katz, L. N. (1959) Amer. J. Physiol. **197,** 955.
Kabins, S. A., Fridman, J., Neustadt, J., Espinosa, G., Katz, L. N. (1960) Amer. J. Physiol. **198**1, 543.
Kahlson, G., (1960) Lancet **1,** 67.
Kahlson, G., Rosengren, E., Thunberg, R. (1966) Lancet **1,** 782.
Kahn, I. S., Stout, B. (1932) J. amer. med. Ass. **99,** 1494.
Kaiser, G. A., Ross, J., Braunwald, E. (1964) J. Pharmacol. exp. Ther. **144,** 156.
Kallos, P., Pagel, W. (1937) Acta med. scand. **91,** 292.
Kämmerer, H. (1928) Z. Hals-Nas.-Ohrenheilk. **20,** 38.
Katz, G. (1940) Science **91,** 221.

Katz, G., Cohen, S. (1941) J. amer. med. Ass. **117,** 1782.
Katz, G. (1942) Proc. Soc. exp. Biol., N.Y. **49,** 272.
Kayser, R. (1895) Arch. int. Laryng. **3,** 101.
Keatinge, W. R., Nadel, J. A. (1965) J. appl. Physiol. **20,** 65.
Keeney, E. L. (1964) J. Allergy **35,** 215.
Keogh, C. A. (1965) In: Diseases of the Ear, Nose and Throat. Ed. Scott-Brown, W. G., Ballantyne, J., Grove, J. (Butterworths, London) p. 62, 75 & 152.
Kern, R. A., Schenk, H. P. (1933) J. Allergy **4,** 485.
Keuning, J. (1963) Int. Rhinol. **1,** 57.
Kim, C. J. (1965) J. Allergy **36,** 353.
Kirkman, W. P. (Quoted by Phoebus, 1862, p. 137).
Klein, S. P., Dishoeck, H. A. E. van (1942) Ned. T. Geneesk. **86,** 2699.
Klein, R. C., Salvaggio, J. E. (1966) J. Allergy **37,** 158.
Knol, K. (1965) Een Klinisch en Epidemiologisch Onderzoek naar de Betekenis van Bronchiale Hyperreactiviteit bij Kinderen met Chronische Aspecifieke Respiratoire Aandoeningen (CARA). (Thesis, Groningen).
Koch, J., Szerb, J. (1950) Arch. int. Pharmacodyn. **81,** 91.
Koch, W., Kaplan, D. (1966) J. Allergy **37,** 102 (Abstract No. 10).
Kontos, H. A., Magee, J. H., Shapiro, W., Patterson, J. L. (1964) Circ. Res. **14,** 351.
Konzett, H. (1940) Arch. exp. Path. Pharmakol. **197,** 27.
Konzett, H. Stürmer, E. (1960) Nature **188,** 998.
Konzett, H. (1963) In: Mode of Action of Drugs. First International Pharmacological Meeting, 1961, Vol. 9, Part 1 & 2. Ed. Uvnäs, B. (Pergamon Press, Oxford) p. 39 & 183.
Koppanyi, T. (1948) Bull. Johns Hopk. Hosp. **83,** 463.
Kottegoda, S. R. (1953) Brit. J .Pharmacol. **8,** 83.
Kottegoda, S. R. (1953) Brit. J. Pharmacol. **8,** 156.
Kovacs, A. (1950) Experientia **6,** 349.
Kragh, J. (1939) Acta oto-laryng., Stockh. Suppl. **51,** 273.
Krajina, Z., Kosokovic, F. (1965) Int. Rhinol. **3,** 81.
Krajina, Z. (1964) Int. Rhinol. **2,** 75.
Kratschmer, F. (1870) Wien. Akad. Sitzungsb. **62,** 147.
Kratz, J. C., Carr, C. J. (1961) Pharmacological Principles of Medical Practice (Williams & Wilkins, Baltimore).
Kraut, H., Frey, E. K., Werle, E. (1930) Z. Physiol. Chem. **189,** 97.
Kreukniet, J. (1959) Longtuberculose en de Asthmatische Constitutie. (Thesis, Goningen).
Kreukniet, J., Young, E. (1964) Med. thorac. **21,** 284.
Krog, J., Leraand, S. (1965) Bibl. anat. **7,** 69.
Krogh, A. (1930) The Anatomy and Physiology of Capillaries (Yale University Press, New Haven).
Krogsaard, A. R. (1958) Acta med. scand. **162,** 465.
Krueger, A. P., Smith, R. F. (1960) J. gen. Physiol. **43,** 533.
Kuhn, H. A., Linton, L. D. (1942) Ann. Otol. Rhinol. Laryng. **51,** 162.
Kuntz, A. (1934) Ann. Otol. Rhinol. Laryng. **43,** 50.
Kuntz, A. (1936) Ann. Otol. Rhinol. Laryng. **45,** 394.
Kuntz, A. (1953) The Autonomic Nervous System. (Lea & Febiger, Philadelphia).
Lake, C. F. (1963) Postgrad. Med. **34,** 306.
Landis, E. M. (1927) Amer. J. Physiol. **81,** 124.
Landis, E. M. (1934) Physiol. Rev. **14,** 404.
Langen, C. D. de (1964) The Peripheral Circulation of Blood, Lymph and Tissue
Langlois, J. P. (1906) Presse méd. **14,** 583.
Fluid (Bohn N.V., Haarlem).
Larsell, O., Fenton, R. A. (1936) Arch. Otolaryng. **24,** 687.
Larsell, O., Barnes, J. F., Fenton, R. (1938) Arch. Otolaryng. **27,** 266.
Laub, G. R. (1945) Laryngoscope **55,** 179.

Laurence, D. R., Moulton, R. (1960) Clinical Pharmacology (Churchill, London).
Lawther, P. J. (1958) Proc. roy. Soc. Med. **51,** 262.
Lecomte, J. (1955) Arch. int. Pharmacodyn. **101,** 375.
Lecomte, J., Petit, J. M. (1960) Int. Arch. Allergy **17,** 10.
Lecomte, J. (1961) Int. Arch. Allergy **18,** 3.
Lecomte, J., Petit, J. M., Mélon, J., Troquet, J., Marcelle, R. (1962) Arch. int. Pharmacodyn. **137,** 232.
Lecomte, J., Baeckeland, E. (1963) In: De Betekenis van de Basophiele Cel, de Eosinophiele Leucocyt en de Lymphocyt voor de Allergologie. Verslag van de Vergadering van de Nederlandse Vereniging voor Allergie, Leiden.
Ledelii, S. (1683) Miscellanea Curiosa (Nuremberg, Dec. II, Annus II), p. 309
Legier, J. E. (1958) Arch. int. Pharmacodyn. **115,** 201.
Lehmann, G. (1939) Arb. Physiol. **10,** 418.
Lehmann, G. (1938) Die Filterung der Atemluft und deren Bedeutung für Staubkrankheiten. (Berlin).
Leicher, H. (1928) Z. Hals-Nas.-Ohrenheilk. **20,** 238.
Lembeck, F. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 147.
LeMessurier, D. H., Schwartz, C. J., Whelan, R. F. (1959) Brit. J. Pharmacol. **14,** 246.
Levinson, L., Beakey, J. F. (1948) Ann. Allergy **6,** 705.
Lewis, G. P. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 26.
Lewis, G. P. (1960) Physiol. Rev. **40,** 647.
Lewis, G. P. (1960) Nature **188,** 999.
Lewis, G. P. (1963) Ann. N.Y. Acad. Sci. **104,** 236.
Lewis, G. P. (1963) In: Mode of Action of Drugs. First International Pharmacological Meeting, 1961. Vol. 9, Part 1. Ed. Uvnäs, B. (Pergamon Press, Oxford) p. 29.
Lewis, J. J. (1960) Introduction to Pharmacology (Livingstone, Edinburgh).
Lewis, T., Grant, R. (1924) Heart **11,** 209.
Lewis, T. (1924) Heart **11,** 199.
Lewis, T. (1926) Heart **13,** 153.
Lewis, T. (1927) Blood Vessels of the Human Skin and their Responses (Shaw, London).
Lewy, B. (1891) Berl. klin. Wschr. **34,** 845.
Lier, J. A. J. van (1960) Een vergelijkende Studie over de Rhinitis Vasomotoria Allergica en Non-Allergica. (Thesis, Leiden).
Lierle, D. M., Moore, P. M. (1934) Arch. Otolaryng. **19,** 55.
Lindell, S. E., Westling, H. (1966) In: Handbook of Experimental Pharmacology, Vol. 18. Histamine and Anti-Histaminics. Part I: Histamine, Its Chemistry, Metabolism and Physiological and Pharmacological Actions. Ed. Eichler, O., Farah, A., Rocha e Silva, M. (Springer-Verlag, Berlin) p. 734.
Lindsay, J. A., Walsh, T. E. (1933) Arch. Otolaryng. **17,** 783.
Litt, M. (1961) J. Immunol. **87,** 522.
Litt, M. (1962) J. Allergy **33,** 532.
Lobitz, W. C., Campbell, C. J. (1953) Arch. Derm. Syph. **67,** 575.
Locke, A. (1939) J. Immunol. **36,** 365.
Lockett, M. F. (1954) Brit. J. Pharmacol. **9,** 498.
Logan, W. P. D., Cushion, A. A. (1958) Studies on Medical and Population Subjects (No. 14, London, H.M.S.O.).
Lowell, F. C., Schiller, F. W. (1948) J. Allergy **19,** 100.
Lowell, F. C. (1956) Amer. J. Med. **20,** 778.
Lowell, F. C. (1964) J. Allergy **35,** 322.
Lucas, A. M. (1935) J. Physiol. **112,** 468.
Lucas, H. A. (1952) J. Laryng. **66,** 480.
Majno, G., Palade, G. E. (1961) J. biophys. biochem. Cytol. **11,** 571.

Majno, G., Palade, G. E., Schoefl, G. T. (1961) J. biophys. biochem. Cytol. **11,** 607.
Malan, A. (1928) Arch. ital. Ot. ecc. **39,** 245.
Malcomson, K. G. (1959) J. Laryng. **73,** 73.
Mandell, M. (1965) In: Abstracts of Symposia. The Sixth European Congress of Allergology, Stockholm. p. 12.
Marshall, R. J. (1959) Progr. cardiovasc. Dis. **1,** 341.
Marshall, R. J., Helmholz, H. F., Shepherd, J. T. (1959) Circulation **20,** 391.
Martinez, L., De Le Tona, J., Castro de la Mata, R., Aviado, D. M. (1961) J. Pharmacol. exp. Ther. **133,** 295.
Mathov, E., Misenta, J. (1963) Ann. Allergy **21,** 481.
Matthews, Le R., Spector, S., Lemm, J., Potter, J. L. (1963) Amer. Rev. resp. Dis. **88,** 199 .
Mautner, H., Pick, E. P. (1915) Münch. bed. Wschr. **62,** 1141.
Mautner, H., Pick, E. P. (1922) Biochem. Z. **127,** 22.
Mautner, H., Pick, E. P. (1928) Arch. exp. Path. Pharmakol. **142,** 271.
Maximow, A. A. (1924) Physiol. Rev. **4,** 533.
Maximow, A. A., Bloom, W. (1952) A Textbook of Histology (Saunders, Philadelphia) p. 27, 30.
McCanon, P. M., Horvath, S. M. (1954) Amer. J. Physiol. **179,** 131.
McCarrell, J. D., Drinker, C. K. (1941) Amer. J. Physiol. **133,** 64.
McCulloch, J. (1828) An Essay on the Remittent and Intermittent Diseases. (London) p. 394.
McDowall, R. J. S. (1947) J. Physiol. **106,** 1.
McGovern, J. P. (1959) Ann. Allergy **17,** 915.
McGraff, C. J., Milnor, W. R. (1960) Fed. Proc. **19,** 96.
McGraff, C. J., Milnor, W. R. (1962) Amer. J. Physiol. **202,** 957.
McGregor, R. C. (1934) J. Physiol. **80,** 65.
McHaffrie, R. A. (1958) J. Allergy **29,** 545.
McIntire, F. C., Roth, L. W., Sproull, M. (1950) Proc. Soc. exp. Biol. **73,** 605
McIntire, F. C., Roth, L. W., Sproull, M. (1951) Amer. J. Physiol. **167,** 233.
McIntosch, F. C., Paton, W. D. H. (1949) J. Physiol. **109,** 190.
McKenzie, J. N. (1884) Amer. J. med. Sci. **87,** 360.
McKlem, P. T., Fraser, R. G., Bates, D. V. (1963) J. appl. Physiol. **18,** 699.
McLaurin, J. W., Shipman, W. F., Kirkley, D. E. (1960) Laryngoscope **70,** 155.
Medical Research Council's Committee on Aetiology of Chronic Bronchitis (1960) Brit. med. J. **II,** 1665.
Melchior, R. (1955) Acta oto-rhino-laryng. belg. **9,** 239.
Mellanby, E., Twort, F. W. (1912) J. Physiol. **45,** 53.
Melmon, K. L., Sjoerdsma, A., Mason, D. T. (1965) Amer. J. Med. **39,** 568.
Mélon, J., Lecomte, J. (1958) Acta allerg. **12,** 43.
Mélon, J. (1963) Acta oto-rhino-laryng. belg. **17,** 437.
Mélon, J. (1964) Acta oto-rhino-laryng. belg. **18,** 1.
Melville, K. I., Shister, H. E. (1962) In: Blood Vessels and Lymphatics. Ed. Abramson, D. I. (Academic Press, New York) p. 77 & 209.
Mendel, H. (1897) Physiologie et Pathologie de la Respiration Nasale. (Paris) [Quoted by Uddströmer, 1940].
Messerklinger, W. (1958) Arch. Ohr.-Nas.-KehlHeilk. **173,** 1.
Meyler, L. (1963) Side Effects of Drugs (Exerpta Medica Found., Amsterdam) p. 91.
Michelson, A. L., Hollander, W., Lowell, F. C. (1958) J. Lab. clin. Med. **51,** 57.
Michgelsen, H. W. B. (1959) In: Inhalatietests en Inhalatie Therapie. Verslag van een Vergadering van de Belgische en Nederlandse Verenigingen voor Allergie (H. E. Stenfert Kroese, Leiden) p. 62.
Middleton, E., Sherman, W. B. (1961) Med. int. Congr. Series **42,** 3.
Miles, A. A. (1951) J. Physiol. **114,** 35P.
Miles, A. A., Miles, E. M. (1952) J. Physiol. **118,** 228.
Millman, M. (1964) Ann. Allergy **22,** 369.

Milloning, A. F., Harris, H. E., Gardner, W. J. (1950) Arch. Otolaryng. **52,** 359.
Mills, A. E. (1956) Laryngoscope **46,** 846.
Mitchell, R. G., Code, C. F. (1954) J. clin. Endocrin. **14,** 707.
Mitchell, R. G., Logan, G. B., Peters, G. A., Henderson, L. L. (1954) J. Allergy **25,** 504.
Mohun, N. (1943) Arch. Otolaryng. **37,** 699.
Moll, H. H. (1940) Quart. J. Med. **9,** 229.
Mongar, J. L., Schild, H. O. (1952) J. Physiol. **118,** 461.
Mongar, J. L. (1952) J. Physiol. **119,** 48P.
Mongar, J. L., Whelan, R. F. (1953) J. Physiol. **120,** 146.
Mongar, J. L. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 74.
Mongar, J. L., Schild, H. O. (1962) Physiol. Rev. **42,** 226.
Moore, D. H., Ruska, H. (1957) J. biophys. biochem. Cytol. **3,** 457.
Moore, J. A. (1960) Nose and Throat Histology (MacMillan, New York).
Moran, N. C. (1959) J. Pharmacol. exp. Ther. **125,** 85.
Moritz, A. R., Henriques, F. C., McLean, R. (1945) Amer. J. Path. **21,** 311.
Moritz, A. R., Weisiger, J. R. (1945) Arch. intern. Med. **75,** 233.
Mota, I., Beraldo, W. T., Junqueira, L. C. U. (1953) Proc. Soc. exp. Biol. **33,** 455.
Mota, I., Berado, W. T., Ferri, A. G., Junqueira, L. C. U. (1954) Nature **174,** 698.
Mota, I., Vugman, I. (1956) Brit. J. Pharmacol. **11,** 304.
Mota, I., Vugman, I. (1956) Nature **177,** 427.
Mota, I. (1958) Nature **182,** 1021.
Mota, I. (1958) J. Physiol. **140,** 6P.
Mota, I., Beraldo, W. T., Ferri, A. G., Junqueira, L. C. U. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 47.
Mota, I., (1959) J. Physiol. **147,** 425.
Mota, I., Dias da Silva, W. (1960) Nature **186,** 245.
Mota, I., Dias da Silva, W. (1960) Brit. J. Pharmacol. **15,** 396.
Mota, I. (1966) In: Handbook of Experimental Pharmacology, Vol. 18. Histamine and Anti-Histaminics. Part I: Histamine, Its Chemistry, Metabolism and Physiological and Pharmacological Actions. Ed. Eichler, O., Farah, A., Rocha e Silva, M. (Springer-Verlag, Berlin) p. 569.
Mount, L. E. (1956) J. Physiol. **131,** 393.
Mudd, S., Goldman, A., Grant, S. B. (1921) J. exp. Med. **34,** 11.
Mudd, S., Grant, S. B., Goldman, A. (1921) J. Lab. clin. Med. **6,** 175, 253 & 322.
Mullin, W. V., Ball R. P. (1928) Ann. Otol. Rhinol. Laryng. **37,** 192.
Murray, N., Bierer, J. (1951) Psyd. Med. **13,** 56.
Myhrman, G., Tomenius, J. (1939) Arch. exp. Path. Pharmakol. **193,** 14.
Nadel, J. A. Widdicombe, J. G. (1962) J. appl. Physiol. **17,** 861.
Nadel, J. A., Salem, H., Tamplin, B., Tokiwa, Y. (1965) J. appl. Physiol. **20,** 164.
Naeye, R. L. (1962) Med. thorac. **19,** 494.
Nahas, G. G. (1958) J. Physiol. **50,** 425.
Nahas, G. G., McDonald, I. (1959) Amer. J. Physiol. **196,** 1045.
Nasmyth, P. A., Stewart, H. C. (1950) J. Physiol. **111,** 19P.
Nef, J. (1962) Schweiz. med. Wschr. **92,** 539.
Negus, V. E. (1951) Acta oto-laryng. Stockh. Suppl. **100,** 74.
Negus, V. E. (1956) Brit. med. J. **I,** 367.
Negus, V. (1957) Ann. Otol. Rhinol. Laryng. **66,** 344.
Neil, J. H., Gilmour, W., Gwynne, F. J., Main, W., Fairclough, W. A. (1937) Ann. Otol. Rhinol. Laryng. **46,** 338.
Nelson, L. S., Stoesser, A. V. (1962) Ann. Allergy **20,** 107.
Netter, F. H. (1962) The Ciba Collection of Medical Illustrations, Volume I. Nervous System. (Ciba).
Neusser, E. (1892) Wien. klin. Wschr. **5,** 41 (Quoted by Hansel, 1934).

Nickerson,, M. (1965) In: Pharmacology of Cholinergic and Adrenergic Transmission. Second International Pharmacological Meeting, Vol. 3. Ed. Koelle, G. B., Douglas, W. W., Carlsson, A., Trcka, V. (Pergamon Press, Oxford). p. 303.
Niekerk, J. van, Leeuwen, S. van (1928) Z. ges. exp. Med. **63,** 393.
Nishiyama, R., Tasaka, K., Irino, S. (1937) Acta med. Okayama **11,** 133 (Quoted by Halpern, 1960).
Noah, J. W., Brand, A. (1957) J. Allergy **28,** 405.
Noon, L. (1911) Lancet **1,** 1572.
Norton, S., Beer, E. J. de (1955) Arch. int. Pharmacodyn. **102,** 352.
Ogden, H. D. (1956) Ann. Allergy **14,** 385.
Ogura, J. H., Stoksted, P. (1958) Laryngoscope **68,** 2001.
Ogura, J. H., Nelson, J. R., Dammkoehler, R., Kawasaki, M., Togawa, K. (1964) Ann. Otol. Rhinol. Laryng. **73,** 381.
Oliver, G., Schäfer, E. A. (1895) J. Physiol. **18,** 230.
Olsen, C. R., Colebatch, H. J. H., Mebel, P. E., Nadel, J. A., Staub, N. C. (1965) J. appl. Physiol. **20,** 202.
O'Neill, D., Malcomson, K. (1954) Brit. med. J. **I,** 554.
Oosterhuis, K. (1951) Ned. T. Geneesk. **95,** 2775.
Opie, E. (1904) Amer. J. med. Sci. **127,** 988.
Ordman, D. (1961) Ann. Allergy **19,** 29.
Orie, N. G. M., Sluiter, H. J., Vries, K. de, Tammeling, G. J., Witkop, J. (1961) In: Bronchitis, An International Symposium. Ed. Orie, N. G. M., Sluiter, H. J. (Van Gorcum, Assen) p. 43.
Ouer, R. A. (1955) Ann. Allergy **13,** 296.
Outschoorn, A. S., Jacob, J. (1960) Brit. J. Pharmacol. **15,** 131.
Page, I. H. (1952) J. Pharmacol. exp. Ther. **105,** 58.
Page, I. H., McCubbin, J. W. (1953) Circ. Res. **1,** 354.
Page, I. H., McCubbin, J. W. (1953) Amer. J. Physiol. **174,** 436.
Page, I. H. (1954) Physiol. Rev. **34,** 563.
Page, I. H., Erspamer, V. (1954) Pharmacol. Rev. **6,** 425.
Page, I. H. (1958) Physiol. Rev. **38,** 277.
Page, I. H. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press. London) p. 93.
Palm, P. E., McNerney, J. M., Hatch, T. (1956) Arch. industr. Hlth. **13,** 55.
Palmer, A. (1935) Ann. Otol. Rhinol. Laryng. **44,** 25.
Panzani, R., Pascal, N. (1961) Int. Arch. Allergy **18,** 199.
Papacostas, C. A., Loew, E. R., West, G. B. (1959) Arch. int. Pharmacodyn. **120,** 353.
Papanicolaou, G. N., Bridges, E. L., Bailey, C. (1961) Amer. Rev. resp. Dis. **85,** 641.
Parratt, J. R., West, G. B. (1957) J. Physiol. **137,** 179.
Parratt, J. R., West, G. B. (1958) J. Physiol. **140,** 105.
Paton, W. D. M. (1951) Brit. J. Pharmacol. **6,** 499.
Paton, W. D. M., Schachter, M. (1951) Brit. J. Pharmacol. **6,** 509.
Paton, W. D. M. (1955) Int. Arch. Allergy **6,** 203.
Paton, W. D. M. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 59.
Paton, W. D. M. (1957) Pharmacol. Rev. **9,** 269.
Paton, W. D. M. (1963) In: Pharmacology of Smooth Muscle. Second International Pharmacological Meeting, 1963, Vol. 6. Ed. Bülbring, B. (Pergamon Press, Oxford) p. 71.
Patterson, R., Chang, W. W. Y., Pruzansky, J. J. (1963) J. Allergy **34,** 455.
Pattle, R. E., Burgess, F. (1957) J. Path. Bact. **73,** 411.
Paulsen, H. C. (1965) In: Abstracts. Sixth European Congress of Allergology, Stockholm. (Ohlssons Boktryckeri, Lund) p. 40.
Pehu, M., Woringer, P. (1933) Acta paediat. **16,** 36.
Peipers, A. (1931) Z. ImmunForsch. **71,** 359.

Pennington, E. S. (1941) J. Allergy **12,** 388.
Percepied, E. (1912) Rev. Laryng., Paris **33,** 417.
Perret, P. (1955) Pract. oto-rhino-laryng. **15,** 243.
Peterson, R. D. A., Page, A. R., Good, R. A. (1962) J. Allergy **33,** 406.
Phoebus, P. (1862) Der Typische Fruhsommer Katarrh oder das sogennante Heufieber, Heu-Asthma. (Giesen).
Platts, M. M., Hammond, J. D., Stuart-Harris, C. H. (1960) Quart. J. Med. **29,** 559.
Policard, A., Galy, P. (1945) Les Bronches (Masson, Paris).
Pollock, H. L. (1919) J. amer. med. Ass. **73,** 591.
Poos, E. E. (1958) Ann. Otol. Rhinol. Laryng. **67,** 1073.
Pratesi, P., Grana, E. (1965) In: Advances in Drug Research. Ed. Harper, N. J., Simmonds, A. B. (Academic Press, London) p. 127.
Prausnitz, C. (1930) Handb. path. Mikroorg. **3,** 125.
Prigal, S. J. (1960) In: Fundamentals of Modern Allergy. Ed. Prigal, S. J. (McGraw-Hill Book Co., New York) p. 81.
Proetz, A. W. (1929) Ann. Otol. Rhinol. Laryng. **38,** 963.
Proetz, A. W. (1932) Ann. Otol. Rhinol. Laryng. **41,** 125 & 1117.
Proetz, A. W. (1933) Ann. Otol. Rhinol. Laryng. **42,** 778.
Proetz, A. W. (1934) Ann. Otol. Rhinol. Laryng. **43,** 450.
Proetz, A. W. (1934) J. Laryng. **49,** 557.
Proetz, A. W. (1939) Arch. Otolaryng. **30,** 509.
Proetz, A. W. (1943) Essays on the Applied Physiology of the Nose (Annals Publ. Co., St. Louis).
Proetz, A. W. (1945) Ann. Otol. Rhinol. Laryng. **54,** 739.
Proetz, A. W. (1948) Ann. Otol. Rhinol. Laryng. **56,** 328.
Quarles van Ufford, W. J. (1954) In: Hooikoorts. Verslag van een Symposium op 8 mei 1954 te Leiden onder Voorzitterschap van Prof. Dr. H. A. E. van Dishoeck georganiseerd door de Nederlandse Vereniging voor Allergie (H. E. Stenfert Kroese, Leiden) p. 52.
Quinlan, P. T. (1950) Brit. med. J. **1,** 1052.
Rackemann, F. M., Tobey, H. G. (1929) Arch. Otolaryng. **9,** 612.
Rackemann, F. M. (1931) Clinical Allergy. Asthma and Hay Fever (MacMillan, New York).
Rackemann, F. M., Simon, F. A. (1935) J. Allergy **6,** 184.
Rackemann, F. M., Weille, F. L. (1939) Arch. Otolaryng. **30,** 1051.
Rackemann, F. M. (1958) J. Allergy **29,** 535.
Raffel, S. (1953) Immunity, Hypersensitivity, Serology (Appleton-Century-Crofts Inc., New York).
Rall, J. E., Gilbert, N. C., Trumph, R. (1945) J. Lab. clin. Med. **30,** 953.
Ralston, H. J., Kerr, W. J. (1945) Amer. J. Physiol. **144,** 305.
Ramsdell, S. G. (1928) J. Immunol. **15,** 305.
Randolph, T. G., Rackemann, F. M. (1941) J. Allergy **124,** 50.
Rappaport, B. Z., Samter, M., Catchpole, H. R., Schiller, F. (1953) J. Allergy **24,** 35.
Rapport, M. M., Green, A. A., Page, I. H. (1948) J. biol. Chem. **176,** 1243.
Rapport, M. M. (1949) J. biol. Chem. **180,** 961.
Ratner, B., Silberman, D. E. (1953) J. Allergy **24,** 371.
Recant, L., Forsham, P. H., Thorn, G. W. (1948) J. clin. Endocrin. **8,** 589.
Redd, L., Vaughn, J. H. (1955) Proc. Soc. exp. Biol. **90,** 317.
Reed, G. F. (1963) Postgrad. Med. **34,** 464.
Reichle, H. S. (1929) J. amer. med. Ass. **92,** 443.
Reid, D. D., Fairbairn, A. S. (1958) Lancet **1,** 1147.
Reid, G. (1952) J. Physiol. **118,** 435.
Reid, G., Rand, M. (1952) Nature **169,** 801.
Renner, W. C. (1961) Ann. Allergy **19,** 903.
Rich, A. R. (1941) Physiol. Rev. **21,** 70.

Rich, H. (1922) Laryngoscope **32,** 510.
Richter, M., Sehon, A. H. (1960) J. Allergy **31,** 111.
Richtner, N. G. (1943) Acta oto-laryng. Suppl. 49.
Ridpath, R. F. (1923) Ann. Otol. Rhinol. Laryng. **32,** 464.
Riggs, D. S. (1963) The Mathematical Approach to Physiological Problems (Williams & Wilkins, Baltimore).
Riker, W. F. (1953) Pharmacol. Rev. **5,** 1.
Riley, J. F., West, G. B. (1953) J. Physiol. **120,** 528.
Riley, J. F., West, G. B. (1955) J. Path. Bact. **69,** 269.
Riley, J. F. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 45.
Riley, J. F. (1959) The Mast Cells (E. & S. Livingstone, Edinburgh).
Ringoen, A. R. (1938) In: Handbook of Hematology. Ed. Downey, H. (Hoeber, New York) p. 179.
Ripe, E. (1965) In: Abstracts of Symposia. The Sixth European Congress of Allergology, Stockholm. p. 19.
Roberts, M., Adam, H. M. (1950) Brit. J. Pharmacol. **5,** 526.
Robin, E., Knowles, J. H. (1962) In: Principles of Internal Medicine. Ed. Harrison, T. R., Adams, R. D., Bennett, I. L., Resnik, W. H., Thorn, G. W., Wintrobe, M. M. (McGraw-Hill Book Co., New York) p. 1574.
Rocha e Silva, M. (1944) J. Allergy **15,** 399.
Rocha e Silva, M., Schild, H. O. (1949) J. Physiol. **109,** 448.
Rocha e Silva, M., Beraldo, W. T., Rosenfeld, G. (1949) Amer. J. Physiol. **156,** 261.
Rocha e Silva, M., Bier, O., Aronson, M. (1951) Nature **168,** 465.
Rocha e Silva, M., Aronson, M. (1952) Brit. J. exp. Path. **33,** 577.
Rocha e Silva, M. (1955) Histamine its Role in Anaphylaxis and Allergy. (Thomas, Springfield).
Rocha e Silva, M., Corrado, A. P., Ramos, A. O. (1960) J. Pharmacol. exp. Ther. **128,** 217.
Rocha e Silva, M. (1961) Chemotherapia **3,** 544.
Rocha e Silva, M. (1966) In: Handbook of Experimental Pharmacology, Vol. 18. Histamine and Anti-Histaminics. Part I: Histamine. Its Chemistry, Metabolism and Physiological and Pharmacological Actions. Ed. Eichler, O., Farah, A., Rocha e Silva, M. (Springer-Verlag, Berlin) p. 431.
Roddie, I. C., Shephard, J. T., Whelan, R. F. (1955) J. Physiol. **130,** 8P.
Roddie, I. C., Shephard, J. T., Whelan, R. F. (1955) Brit. J. Pharmacol. **10,** 445.
Rokaw, S. N., Massey, F. (1962) Amer. Rev. resp. Dis. **86,** 703.
Rosa, L., Cenacchi, G. C., Berami, G. (1958) Acta allerg. **12,** 427.
Rose, B. (1941) J. Immunol. **42,** 161.
Rose, B. (1941) J. clin. Invest. **20,** 419.
Rose, B. (1941) J. Allergy **12,** 327.
Rose, B., Browne, J. S. L. (1941) Amer. J. Physiol. **131,** 589.
Rose, B. (1947) Amer. J. Med. **3,** 545.
Rose, B. (1954) J. Allergy **25,** 168.
Rose, J. C. (1957) Circulation **16,** 931.
Rose, J. C. (1957) J. clin. Invest. **36,** 924.
Rose, J. C., Lazaro, E. J. (1958) Circ. Res. **6,** 283.
Rosenblueth, A., Simeon, E. F. A. (1938) Amer. J. Physiol. **122,** 708.
Rosenthal, G. (1904) Presse méd. No. 129 & 177.
Rosenthal, S. M., Tabor, H. J. (1948) J. Pharmacol. exp. Ther. **92,** 425.
Rothschild, A. M. (1966) In: Handbook of Experimental Pharmacology, Vol. 18. Histamine and Anti-Histaminics. Part I: Histamine. Its Chemistry, Metabolism and Physiological and Pharmacological Actions. Ed. Eichler, O., Farah, A., Rocha e Silva, M. (Springer-Verlag, Berlin) p. 386.
Rowe, A. H. (1937) Clinical Allergy due to Foods, Inhalants, Contactants, Fungi, Bacteria and Other Causes (Lea & Febiger, Philadelphia).
Rudolph, A. M., Paul, M. H. (1957) Fed. Proc. **16,** 110.

Rudolph, A. M. (1962) Med. thorac. **19,** 376.
Rudolph, J., Cohen, M. (1934) J. Allergy **5,** 476.
Ruskin, S. L. (1925) Laryngoscope **35,** 87.
Rytömaa, T., Teir, H. (1961) Nature **192,** 271.
Rytömaa, T. (1962) Blood **19,** 439.
Salter, H. H. (1864) On Asthma; Its Pathology and Treatment (Blanchard & Lea, Philadelphia).
Salter, W. T., White, M. L. (1949) Anesthesiology **10,** 553.
Saltzman, H. A., Heyman, A., Sieker, H. O. (1963) New Engl. J. Med. **268,** 1431.
Salvato, G. (1961) Int. Arch. Allergy **18,** 348.
Samet, P., Bernstein, W. H., Widrich, J. (1960) Amer. Heart J. **60,** 433 .
Samter, M. (1949) Blood **4,** 217.
Samter, M., Erickson, E. E., Kofoed, M. A. (1952) J. Allergy **23,** 40.
Samter, M., Kofoed, M. A., Pieper, W. (1953) Blood **8,** 1078.
Samter, M. (1955) J. Allergy **26,** 219.
Samuel, S. (1890) Arch. Path. **121,** 396.
Sandmann, G. (1893) Verh. laryng. Ges. Berl. (November).
Sanyal, R. K. (1961) Int. Arch. Allergy **18,** 197.
Sauer, W. G., Dearing, W. H., Flock, E. V. (1958) J. amer. med. Ass. **168,** 139.
Schachter, M., Talesnik, J. (1952) J. Physiol. **118,** 258.
Schachter, M. (1952) Brit. J. Pharmacol. **7,** 646.
Schachter, M. (1956) Brit. J. Pharmacol. **11,** 111.
Schachter, M. (1960) In: Polypeptides which affect Smooth Muscles and Blood Vessels. Ed. Schachter, M. (Pergamon Press, London).
Schachter, M. (1963) In: Mode of Action of Drugs. First International Pharmacological Meeting, 1961, Vol. 9, Part 1. Ed. Uvnäs, B. (Pergamon Press, Oxford) p. 87.
Schaeffer, J. P. (1920) The Nose, Paranasal Sinuses. (Blakiston, Philadelphia).
Schayer, R. W., Davis, K. J., Smiley, R. L. (1955) Amer. J. Physiol. **182,** 54.
Schayer, R. W. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 183.
Schayer, R. W. (1963) Progress in Allergy, Vol. 7. Ed. Kallós, P., Waksman, B. H. (Karger, Basel) p. 187.
Schayer, R. W. (1966) In: Handbook of Experimental Pharmacology, Vol. 18. Histamine and Anti-Histaminics. Part I: Histamine, Its Chemistry, Metabolism and Physiological and Pharmacological Actions. Ed. Eichler, O., Farah, A., Rocha e Silva, M. (Springer-Verlag, Berlin) p. 688.
Scheideler, J. (1938) Z. Hals-Nas.-Ohrenheilk. **44,** 228.
Scheideler, J. (1939) Arch. Ohr.-Nas.-KehlHeilk. **146,** 133.
Scheideler, J. (1939) Arch. Ohr.-Nas.-KehlHeilk. **146,** 170.
Schenk, H. P. (1949) Arch. Otolaryng. **49,** 48.
Scheppegrell, W. (1922) Hay Fever and Asthma (Lea & Febiger, Philadelphia).
Schild, H. O. (1949) Nature **164,** 24.
Schild, H. O., Hawkins, D. F., Mongar, J. L., Herxheimer, H. (1951) Lancet **2,** 376.
Schiller, J., Lowell, J. (1952) J. Allergy **23,** 234.
Schiratzki, H. (1964) Acta oto-laryng. **58,** 535.
Schleinzer, R. (1951) Acta allerg. **4,** 7.
Schmidt, A. D. (1891) Zbl. inn. klin. Med. **12,** 473.
Schnyder, U. W. (1960) Int. Arch. Allergy Suppl. 17.
Schuster, S. A. (1937) Ann. Otol. Rhinol. Laryng. **46,** 124.
Schwarz, E. (1914) Ergebn. Path. **17,** 137.
Schweizer, M. (1953) Endocrinology **53,** 293.
Scott, J. H. (1954) J. Laryng. **68,** 308.
Seebohm, P. M., Hamilton, W. K. (1958) J. Allergy **29,** 56.
Seebohm, P. M. (1965) In: Current Therapy. Ed. Conn, H. F. (Saunders, Philadelphia) p. 418.
Segal, M. S., Beakey, J. F. (1947) Ann. Allergy **5,** 317.

Segal, M. S., Hershfus, J. A., Bresnick, E. (1951) Ann. Allergy **9,** 796.
Segal, M. S., Chodosh, S., Dulfano, M. J. (1965) In: Current Therapy. Ed. Conn, H. F. (Saunders, Philadelphia) p. 83.
Seiffert, O., Kahn, M. (1895) Atlas der Histopathologie der Nase. (Bergman, Wiesbaden).
Selfridge, G. (1920) Laryngoscope **30,** 611.
Semerák, A. (1958) Z. Laryng. Rhinol. **37,** 248.
Serafini, P. (1950) La pollinosi. E.M.E.S., Roma, (Quoted by Orie et al. 1961).
Serafini, U. (1966) In: Handbook of Experimental Pharmacology, Vol. 18. Histamine and Anti-Histaminics. Part I: Histamine, Its Chemistry, Metabolism and Physiological and Pharmacological Actions. Ed. Eichler, O., Farah, A., Rocha e Silva, M. (Springer-Verlag, Berlin) p. 899.
Sewall, E. C., Hunnicutt, M. D. (1929) Arch. Otolaryng. **10,** 1.
Shambaugh, G. E. (1931) Arch. Otolaryng. **13,** 556.
Shambaugh, G. E. (1945) Ann. Otol. Rhinol. Laryng. **54,** 43.
Shanes, A. M. (1965) In: Pharmacology of Cholinergic and Adrenergic Transmission. Second International Pharmacological Meeting, Vol. 3. Ed. Koelle, G. B., Douglas, W. W., Carlsson, A., Trcka, V. (Pergamon Press, Oxford) p. 73.
Sharp, J. T., Paul, O., Lepper, M. H., McKlean, H., Saxton, G. A. (1965) Amer. Rev. resp. Dis. **91,** 510.
Sheffer, A. L., Miller, M. M. (1962) Ann. Allergy **20,** 90.
Shepherd, J. T., Donald, D. E., Linder, E., Swan, H. J. C. (1959) Amer. J. Physiol. **197,** 963.
Sherman, W. B. (1947) Amer. J. Med. **3,** 586.
Sherman, W. B., Kessler, W. R. (1957) Allergy in Pediatric Practice (Mosby, St. Louis).
Sherman, W. B. (1960) In: Fundamentals of Modern Allergy. Ed. Prigal, S. J. (McGraw-Hill Book Co., New York) p. 12.
Shilkret, H. H. (1949) Psychosom. Med. **11,** 127.
Shimamoto ,T. (1964) Bibl. anat. **7,** 271.
Shore, P. A., Carlsson, A., Brodie, B. B. (1956) Fed. Proc. **15,** 483.
Shore, P. A., Pletscher, A., Tomich, E. G., Kuntzman, R., Brodie, B. B. (1956) J. Pharmacol. exp. Ther. **117,** 232.
Shorley, P. G., Collier, H. O. J. (1960) Nature 188, 999.
Short, D. S. (1962) Med. thorac. **19,** 411.
Shulman, R. (1963) Ann. Allergy **21,** 349.
Sice, J. (1962) General Pharmacology (Saunders, Philadelphia).
Sicuteri, F. (1964) Bibl. anat. **7,** 591.
Silberman, D. E., Sorrell, A. H. (1959) J. Allergy **30,** 11.
Simon, F. A., Rackemann, F. M. (1934) J. Allergy **5,** 439.
Sinha, Y. K., West, G. B. (1953) J. Pharm. Pharmacol. **5,** 370.
Sjoerdsma, A., Waalkes, T. P., Weissbach, H. (1957) Science **125,** 1202.
Skalpe, I. O. (1964) Brit. J. industr. Med. **21,** 69.
Slome, D. (1957) Sci. basis Med. **5,** 451.
Slome, D. (1965) In: Diseases of the Ear, Nose and Throat. Ed. Scott-Brown, W. G., Ballantyne, J., Groves, J. (Butterworths, London) p. 24 & 435.
Sluder, G. (1919) J. amer. med. Ass. **73,** 589.
Smellie, H., Fry, L. (1962) Acta allerg. **17,** 352.
Smiley, D. F. (1929) Amer. J. Hyg. **9,** 477.
Smith, D. E. (1958) Proc. Soc. exp. Biol., N.Y. **97,** 872.
Smith, D. E. (1958) Amer. J. Physiol. **193,** 573.
Smith, D. E. (1958) Science **128,** 207.
Smith, R. P., Wagman, W., Riopelle, A. J. (1956) J. Pharmacol. **117,** 136.
Snider, G. L., Barnett, H. K., Radner, D. B., Mosko, M. M. (1955) J. Lab. clin. Med. **46,** 348.
Söderholm, B., Werkö, L. (1959) Brit. Heart J. **21,** 1.

Sollmann, T. (1948) A Manual of Pharmacology and its Applications to Therapeutics and Toxicology (Saunders, Philadelphia).
Solomon, W. R., McLean, J. A., Cookingham, C., Ahronheim, G., DeMuth, G. R. (1965) J. Allergy **36**, 62.
Spain, D. M. (1959) Amer. Rev. Tuberc. **79**, 591.
Spain, W. C., Cooke, R. A. (1924) J. Immunol. **9**, 521.
Sparrow, E. M., Wilhelm, D. L. (1957) J. Physiol. **137**, 51.
Spector, W. G. (1958) Pharmacol. Rev. **10**, 475.
Spector, W. G., Willoughby, D. A. (1965) In: The Inflammatory Process. Ed. Zweifach, B. W., Grant, L., McCluskey, R. T. (Academic Press, New York) p. 427.
Speirs, R. S., Meyer, R. K. (1949) Endocrinology **45**, 403.
Speirs, R. S. (1952) Blood **7**, 550.
Speirs, R. S. (1955) Ann. N.Y. Acad. Sci. **59**, 706.
Speirs, R. S., Wenck, V. (1956) Blood **11**, 56.
Speirs, R. S. (1958) Nature, **181**, 681.
Speizer, F. E., Ferris, B. G. (1963) Amer. Rev. resp. Dis. **88**, 205.
Speizer, F. E., Frank, N. R. (1966) Brit. J. industr. Med. **23**, 75.
Spiesman, I. G. (1936) Amer. J. Physiol. **115**, 181.
Spiess, G. (1900) Heymann's Handb. d. Laryng. u. Rhin. **3** (1), 215.
Spiess, G. (1902) Int. Zbl. Laryng. **18**, 110.
Stacey, R. S. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 116.
Stacey, R. S. (1966) In: Handbook of Experimental Pharmacology, Vol. 19: 5-Hydroxytryptamine and Related Indolealkylamines. Ed. Eichler, O., Farah, A., Erspamer, V. (Springer-Verlag, Berlin) p. 744.
Stalker, W. W., Dickerson, R. C., Kramer, G. D. (1963) Amer. Hyg. Ass. J. **24**, 68 [In: Bull. Hyg. (1963) **38**, 893].
Starr, I. (1933) Amer. J. med. Sci. **186**, 330.
Staub, N. C. (1962) In: Blood Vessels and Lymphatics. Ed. Abramson, D. I. (Academic Press, New York) p. 299.
Steiner, F. A., Hedinger, C. (1956) Experientia **12**, 109.
Steinmann, O. (1948) Pract. oto-rhino-laryng. **10**, 339.
Sternberg, H. (1929) Mschr. Ohrenheilk. **63**, 390.
Sternberg, L. (1928) Amer. J. med. Sci. **176**, 82.
Sternstein, H. J., Schur, M. O. (1936) Arch. Otolaryng. **23**, 475.
Stevens, F. A. (1934) J. Allergy **5**, 285.
Stevens, F. A. (1953) J. Allergy **24**, 446.
Stevenson, R. C., Guthrie, D. (1949) A History of Oto-Laryngology (Livingstone, Edinburgh).
Stoksted, P. (1952) Acta oto-laryng., Stockh. **42**, 175.
Stoksted, P. (1953) Acta oto-laryng., Stockh. Suppl. 109, p. 143, 159.
Stoksted, P., Thomsen, K. A. (1963) Acta oto-laryng., Stockh. Suppl. 109, 176.
Stoksted, P. (1956) Acta oto-laryng. **46**, 64.
Stoksted, P., Nielsen, J. Z. (1957) Acta oto-laryng. Suppl. 140, 236.
Stone, C. A., Wenger, H. C., Ludden, C. T., Stavorski, J. M., Ross, C. A. (1961) J. Pharmacol. exp. Ther. **131**, 73.
Strandberg, L. G. (1964) Arch. environm. Hlth. **9**, 160.
Stresemann, E. (1963) Acta allerg. **18**, 235.
Strömme, O. (1952) Acta oto-laryng., Stockh. Suppl. 104.
Surber, J. R. (1956) Arch. Oto laryng. **64**, 913.
Swindle, P. F. (1935) Ann. Otol. Rhinol. Laryng. **44**, 913.
Swindle, P. F. (1937) Ann. Otol. Rhinol. Laryng. **46**, 600.
Swineford, ●. (1935) J. Allergy **6**, 175.
Szilágyi, T., Kover, A., Csaba, B. (1960) Amer. J. Physiol. **199**, 272.
Takamine, J. (1901) Ther. Gaz. **27**, 221.
Targow, A. M. (1955) Ann. Allergy **13**, 662.
Tatum, A. L. (1923) Amer. J. Physiol. **65**, 229.

Taylor, I., Knowelden, J. (1964) Principles of Epidemiology (Churchill, London).
Taylor, M. (1961) J. Laryng. **75,** 972.
Taylor, M. (1961) J. Laryng. **75,** 1048.
Teatini, G. Massara, G. (1959) Z. Laryng. Rhinol. **38,** 605.
Telford, J. M. West, G.B. (1960) Brit. J. Pharmacol. **15,** 532.
Telford, J. M., West, G. B. (1961) Brit. J. Pharmacol. **16,** 360.
Thompson, J. A. (1919) J. amer. med. Ass. **73,** 587.
Thorn, G. W., Forsham, P. H., Prunty, F. T., Hills, A. G. (1948) J. amer. med. Ass. **137,** 1005.
Thorn, G. W. (1953) Amer. J. Med. **14,** 139.
Thorson, A. (1958) Acta med. scand. Suppl. 161.
Tiffeneau, R. (1955) Sem. Hôp. Paris **31,** 117.
Tiffeneau, R. (1955) Presse méd. **63,** 227.
Tiffeneau, R. (1957) L'Examen Pulmonaire de l'Astmatique (Masson, Paris).
Tiffeneau, R. (1957) Dis. Chest. **31,** 404.
Tiffeneau, R. (1958) Sem. Hôp. Paris **34,** 421.
Tiffeneau, R. (1958) Presse méd. **66,** 1250.
Tiffeneau, R. (1960) Int. Arch. Allergy **17,** 193.
Tiffeneau, R. (1961) Changes (increase or decreacse) in the Degree of Pulmonary Allergy caused by Repeated Inhalation of Allergens. European Academy of Allergy. (H. E. Stenfert Kroese, Leiden).
Tillotson, R. S. (1929) Arch. Otolaryng. **10,** 262.
Toh, C. C. (1957) J. Physiol. **138,** 488.
Toigo, A., Imarisio, J. J., Murmall, H., Lepper, M. N. (1963) Amer. Rev. resp. Dis. **87,** 487.
Tomori, Z., Gombos, B., Benicky, L., Mokránova, L., Luptákova, M. (1964) In: Drugs and Respiration. Second International Pharmacological Meeting. Vol. 2. Ed. Aviado, D. M. Palecek, F. (Pergamon Press, Oxford) p. 221.
Tonndorf, J. (1939) Arch. Ohr.-Nas.-KehlHeilk. **146,** 41.
Tonndorf, J. (1958) Ann. Otol. Rhinol. Laryng. **67,** 984.
Townley, R. G., Macey, D., Itkin, I. H. (1965) J. Allergy **36,** 121.
Trapani, I. L., Szentivanyi, A. (1966) J. Allergy **37,** 101 (Abstracts).
Tremble, G. E. (1948) Laryngoscope **58,** 206.
Trenchard, H. J., Harris, W. C. (1963) Lancet, **1,** 404.
Triggle, D. J. (1965) Chemical Aspects of the Autonomic Nervous System (Academic Press, London).
Troescher-Elam, E., Ancona, G., Kerr, W. (1945) Amer. J. Physiol. **144,** 711.
Tromp, S. W. (1965) Int. Rhinol. **3,** 43.
Tschalussow, M. A. (1913) Pflügers Arch. ges. Physiol. **151,** 523.
Tschalussow, M. A. (1913) Zbl. Biochem. Biophys. **15,** 338.
Tuft, L. (1937) Clinical Allergy (Saunders, Philadelphia).
Tuft, L., Blumstein, G. (1950) J. Allergy **21,** 326.
Tuft, L., Torsney, P. J., Ettelson, L. N., Heck, V. M. (1962) J. Allergy **33,** 448.
Turnbull, J. A. (1920) Boston med. sci. J. **182,** 493.
Uddströmer, M. (1940) Acta oto-laryng., Stockh. Suppl. 42.
Udenfriend, S. (1958) In: 5-Hydroxytryptamine. Ed. Lewis, G. P. (Pergamon Press, London) p. 36.
Underhill, W. L., Tredway, J. B. (1964) Ann. intern. Med. **60,** 680.
Urbach, E. (1933) Klin. Wschr. **83,** 761.
Urbach, E. (1941) Arch. Otolaryng. **33,** 982.
Urbach, E., Gottlieb, P. M. (1946) Allergy (Grune & Stratton, New York).
Uvnäs, B. (1958) J. Pharm. Pramacol. **10,** 1.
Uvnäs, B., Thon, I. L. (1959) Exp. Cell. Res. **18,** 512.
Vaheri, E. (1956) Acta allerg. **10,** 203.
Vail, H. H. (1932) Ann. Otol. Rhinol. Laryng. **41,** 837.
Valentine, W. N., Lawrence, J. S., Pearce, M. L., Beck, W. S. (1955) Blood **10,** 154.

Vallery-Radot, P., Heimann, V. (1930) Hypersensibilités Specifiques dans les Affections Cutanées (Masson, Paris).
Varekamp, H., Voorhorst, R. (1965) Int. Rhinol. **3,** 73.
Vaughan, W. T. (1922) Epidemiology and Public Health (Mosby, St. Louis).
Vaughan, W. T. (1923) J. amer. med. Ass. **80,** 245.
Vaughan, W. T. (1928) Science **68,** 1.
Vaughan, W. T. (1928) J. Lab. clin. Med. **13,** 633.
Vaughan, W. T. (1934) Allergy and Applied Immunology (Kimpton, London).
Vaughn, J. (1952) J. Path. Bact. **64,** 91.
Vaughn, J. (1953) Blood **8,** 1.
Vaughan, W. T., Black, J. H. (1954) Practice of Allergy (Mosby, St. Louis).
Veer, A. van der, Cooke, R. A., Spain, W. C. (1927) Amer. J. med. Sci. **174,** 101.
Vercauteren, R., Peeters, G. (1952) Arch. int. Pharmacodyn. **89,** 10.
Vercauteren, R. (1953) Enzymologia **16,** 1.
Vialli, M. (1966) In: Handbook of Experimental Pharmacology, Vol. 19: 5-Hydroxytryptamine and Related Indolealkylamines. Ed. Eichler, O., Farah, A., Erspamer, V. (Springer-Verlag, Berlin) p. 1.
Vischer, B., Halberg, F. (1955) Ann. N.Y. Acad. Sci. **59,** 834.
Vitolo, E., Obbiassi, M., Zocche, G. P., Scotti, G. C. (1962) Brit. Heart J. **24,** 422.
Volkenburgh, V. A. van, Frost, W. H. (1933) Amer. J. Hyg. **17,** 122.
Voltolini, R. (1880) Allg. med. centr. - Ztg. p. 126.
Voorhorst, R., Vermey-Scheltema, G. C., Varekamp, H. (1960) Ned. T. Geneesk. **104,** 1263.
Voorhorst, R. (1961) In: Allergy. Advances in Oto-Rhino-Laryngology. VII. International Congress. Ed. Dishoeck, H. A. E. van (Karger, Basel) p. 86.
Voorhorst, R. (1962) Basic Facts of Allergy (H. E. Stenfert Kroese, Leiden).
Voorhorst, R. (1963) In: De Betekenis van de Basophiele Cel, de Eosinophiele Leucocyt en de Lymphocyt voor de Allergologie. Verslag van de Vergadering van de Nederlandse Vereniging voor Allergie (Leiden).
Voorhorst, R. (1963) Int. Rhinol. **1,** 65.
Voorhorst, R. (1965) Int. Rhinol. **3,** 65.
Voorhorst, R. (1965) Ned. T. Geneesk. **109,** 376.
Voorhorst, R. (1965) Ned. T. Geneesk. **109,** 425.
Voorhorst, R. (1965) Ned. T. Geneesk. **109,** 475.
Vries, K. de (1961) In: Bronchitis, An International Symposium. Ed. Orie, N. G. M., Sluiter, H. J. (Van Gorcum, Assen) p. 221.
Vries, K. de, Goei, J. T., Booij-Noord, H., Orie, N. G. M. (1962) Int. Arch. Allergy **20,** 93.
Vries, K. de, Orie, N. G. M., Goei, J. T., Booij-Noord, H., Zuiderweg, A. (1963) Bronches **13,** 263.
Vries, K. de, Booij-Noord, H., Goei, J. T., Orie, N. G. M. (1963) In: Proceedings of the Fifth European Congress of Allergy. (Schwabe Basel) p. 316.
Vries, K. de, Booij-Noord, H., Goei, J. T., Grobler, N. J., Sluiter, H. J., Tammeling, G. J., Orie, N. G. M. (1964) In: Bronchitis, Second International Symposium. Ed. Orie, N. G. M., Sluiter, H. J. (Van Gorcum, Assen) p. 167.
Waaler, B. A. (1961) J. Physiol. **157,** 475.
Waalkes, T. P., Weissbach, H., Bozicevich, J., Udenfriend, S. (1957) J. clin. Invest. **36,** 1115.
Waalkes, T. P., Coburn, H., Terry, L. L. (1959) J. Allergy **30,** 408.
Waalkes, T. P., Coburn, H. (1960) J. Allergy **31,** 151.
Waalkes, T. P., Coburn, H. (1960) J. Allergy **31,** 181.
Waalkes, T. P., Coburn, H. (1960) J. Allergy **31,** 395.
Waelen, M. J. G. A. (1963) Vaatverwijdende Middelen (Thesis, Nijmegen).

Wal, A. M. van der (1964) Chronische Aspecifieke Respiratoire Aandoeningen (CARA) als Voorwaarde voor het Ontstaan van het Bronchuscarcinoom (Thesis, Groningen).
Walker, I. C. (1916) J. med. Res. **35**, 497.
Walker, I. C. (1920) J. amer. med. Ass. **75**, 782.
Walker, J. E. C., Wells, R. E. (1961) Amer. J. Med. **30**, 259.
Waller, A. (1846) Phil. Mag (4) **29**, 397.
Walsh, T. D. (1945) Rhinitis in Diseases of the Nose, Throat and Ear (Saunders, Philadelphia).
Walsh, T. E., Lindsay, J. R. (1934) Arch. Otolaryng. **20**, 649.
Walsh, T. E. (1950) Laryngoscope **60**, 360.
Walzer, M. (1938) J. Allergy **10**, 72.
Wearn, J. T., Ernstene, A. C., Bromer, A. W., Barr, J. S., German, W. J., Zschiesche, L. J. (1934) Amer. J. Physiol. **109**, 236.
Weder, A. (1962) Diagnostik und Therapie der Allergischen und Extra-allergischen vasomotorischen Rhinopathie (Karger, Basel).
Weichardt, (1907) Klin. Wschr. **44**, 678.
Weille, F. L. (1930) Arch. Otolaryng. **12**, 785.
Weille, F. L., Gohd, R. S. (1956) Ann. Otol. Rhinol. Laryng. **65**, 443.
Weissbach, H., Waalkes, T. D., Undenfriend, S. (1957) Science **125**, 235.
Weller, H. H. (1965) Bijnierschorsactiviteit bij CARA (Astma) (Thesis, Groningen).
Werle, E., Berek, U. (1948) Angew. Chem. **60A**, 53.
West, G. B. (1956) In: Histamine. Ciba Foundation Symposium. Ed. Wolstenholme, G. E. W., O'Connor, C. M. (Churchill, London) p. 14.
West, G. B. (1957) Int. Arch. Allergy **10**, 257.
West, G. B. (1957) Int. Arch. Allergy **11**, 312.
West, G. B. (1961) Int. Arch. Allergy, **18**, 56.
West, J. B. (1962) Med. thorac. **19**, 392.
Westling, H. (1963) In: Mode of Action of Drugs. First International Pharmacological Meeting, 1961, Vol. 9, Part 2. Ed. Uvnäs, B. (Pergamon Press, Oxford) p. 117.
Wharton-Jones, T. (1851) Guy's Hosp. Rep. **14**, 1.
Whittaker, V. P. (1951) Physiol. Rev. **31**, 312.
Widdicombe, J. G. (1963) Brit. med. Bull. **19**, 15.
Widdicombe, J. G. (1966) In: Advances in Respiratory Physiology. Ed. Caro, C. G. (Edward Arnold Publ., London) p. 48.
Wijk, A. M. van, Patterson, N. S. (1940) J. industr. Hyg. Toxicol. **22**, 31.
Wilhelm, D. L. (1962) Pharmacol. Rev. **14**, 251.
Wilhelm, D. L. (1965) In: The Inflammatory Process. Ed. Zweifach, B. W., Grant, L., McCluskey, R. T. (Academic Press, New York) p. 389.
Williams, H. L. (1958) Ann. Otol. Rhinol. Laryng. **67**, 676.
Williams, R. I. (1956) Ann. Otol. Rhinol. Laryng. **65**, 171.
Wilson, G. S., Miles, A. A. (1964) Principles of Bacteriology and Immunity (Edward Arnold Publ., London).
Wilson, A., Schild, H. O. (1959) Applied Pharmacology (Churchill, London).
Wiman, L. G. (1962) Acta oto-laryng., Stockh. Suppl. 158.
Winbury, M. M. (1964) In: Advances in Pharmacology. Ed. Garatini, S., Shore, P. A. (Academic Press, New York) p. 2.
Winkelmann, R. K. (1961) In: Blood Vessels and Circulation (Advances in biology of Skin) Vol. 2. Ed. Montagna, W., Ellis, R. A. (Pergamon Press, Oxford) p. 1.
Winslow, C. E. A., Greenburg, L., Herrington, L. P. (1934) Amer. J. Hyg. **20**, 195.
Winkenwerder, W. L., Gay, L. N. (1937) Bull. Johns Hopk. Hosp. **61**, 90.
Wit, G. de, Kaptein, T. S., Bochove, W. M. (1965) Int. Rhinol. **3**, 37.
Wit, G. de (1965) Ned. T. Geneesk. **109**, 595.
Withers, O. R., Hale, R. (1956) Ann. Allergy **14**, 384.

Wodehouse, R. P. (1917) J. Immunol. **2,** 227.
Wolf, S., Holmes, T. H., Trenting, T., Goodell, H., Wolff, H. S. (1950) J. Allergy **21,** 1.
Wolf, S. (1954) Arch. Otolaryng. **59,** 461.
Wolff-Eisner, A. (1906) Das Heufieber. (München).
Wood, C. H., Meadows, S. H. (1963) Brit. med. J. **II,** 1088.
Wood, P., Besterman, E. M., Towers, M. K., McIlroy, M. B. (1957) Brit. Heart J. (1957) **19,** 279.
Wood, P. (1958) Brit. Heart J. **20,** 557.
Woodward, F. D., Swineford, O. (1941) Arch. Otolaryng. **34,** 1123.
Woolley, D. W., Shaw, E. (1953) J. biol. Chem. **203,** 69.
Woolley, D. W. (1958) Proc. nat. Acad. Sci., Wash. **44,** 197.
Woolley, D. W. (1958) Proc. nat. Acad. Sci., Wash. **44,** 1202.
Worms, G., Bolotte, M. (1928) L'Insuffisance Respiratoire Nasale. (Paris).
Wright, G. W. (1961) Bact. Rev. **25,** 219.
Wright, J. (1895) Amer. J. med. Sci. **109,** 516.
Wright, J. (1914) A History of Laryngology and Rhinology (Lea & Febiger, Philadelphia).
Wright, P. G. (1964) Bibl. anat. **7,** 294.
Wyman (1872) Autumnal Catarrh. (New York).
Young, I. M. (1957) J. Physiol. **137,** 374.
Yu, P. N. (1962) Med. thorac. **19,** 383.
Zaid, G., Beall, G. N. (1966) J. Allergy **37,** 101 (Abstracts).
Zarniko, C. (1910) Die Krankheiten der Nase und des Nasenrachens. (Berlin).
Ziegelman, E. F. (1934) Ann. Otol. Rhinol. Laryng. **43,** 1091.
Zohman, L., Williams, M. H., Valhalla, N.Y. (1958) J. Allergy **29,** 72.
Zwaardemaker, H. (1889) Ned. T. Geneesk. **1,** 6.
Zwaardemaker, H. (1889) Ned. T. Geneesk. **1,** 297.
Zweifach, B. W. (1953) In: Mechanism of Inflammation. (Acta, Montreal).
Zweifach, B. W. (1961) Functional Behaviour of the Microcirculation. (Thomas Publisher, Springfield).